2007
VOLUME 2
NUMBER 4

FOR LIFE SCIENCE RESEARCH

INNOVATION @ WORK

ABC TRANSPORTERS AND CANCER DRUG RESISTANCE

NF-κB AND INFLAMMATION

DNA DAMAGE AND REPAIR

NEW PRODUCTS

Cultured HeLa cells in metaphase stage of mitosis.

Cellular Mechanisms and Cancer

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Introduction

The medical definition of cancer appears simple and straightforward. According to the U.S. National Cancer Institute, cancer is "(a) term for diseases in which abnormal cells divide without control."[1] Behind this basic definition is a complex and unpredictable spectrum of over 100 types of cancer.

The human aspect of cancer cannot be completely separated from the scientific research. The World Health Organization recognizes cancer as a leading cause of death worldwide, and emphasizes prevention and early detection as crucial to reducing the global burden of the disease.[2] The American Association for Cancer Research established its Scientist-Survivor program in 1999 to encourage communication between patients, patient advocates, and leading scientists in the field.[3] Newspapers, magazines, and other media sources announce breakthrough discoveries to the public, increasing awareness that cancer is not an individual disease but a collection that has no single initiating event or defined evolution.

Cancer results from a cascade of abnormal cell reactions. When a cellular mechanism goes wrong, the resulting damage, if not repaired, may contribute to a cell's evolution into malignancy. Because cancers begin with a single cell, cancer investigators use genomics, proteomics, and signaling techniques to determine and evaluate cellular changes and contributing cause and effect. Discoveries such as the correlation between the human papilloma virus and cervical cancer encourage the scientific community to seek similar breakthroughs for other cancer types. The understanding of cellular mutations and signaling pathways involved in mutagenesis and abnormal cell function has been used to screen potential new drugs with more efficacy and/or less toxicity.

It's impossible to comprehensively review current cancer research; the amount of information is enormous and the rate of discovery is increasing. In the preface to his book "The Biology of Cancer", Robert Weinberg writes "...we are deluged with a vast amount of genetic, biochemical, and cell biological information about cancer development, far more almost than any human mind can assimilate and comprehend."[4] For this issue of BioFiles we have selected three aspects of cancer biology to review.

- The exploitation of ABC transporter proteins by cancer cells to export chemotherapeutic drugs
- The activation of NF–κB in response to inflammation and its role in cancer progression
- Genetic damage, mutagenesis, and cellular repair processes

Innovation @ Work - Innovative products from Sigma for genomics and proteomics studies, including gene silencing, protein expression profiling, phosphopeptide enrichment, and whole genome amplification, are also highlighted.

References:

1. World Health Organization, Cancer Fact Sheet No. 297, Feb. 2006, www.who.int/mediacentre/factsheets/fs297/en/index.html
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3. American Association for Cancer Research, www.aacr.org
4. *The Biology of Cancer,* Robert A. Weinberg, Garland Science, Taylor & Francis Group, LLC, New York, NY (2007)

Cover image: Cultured HeLa cells labeled with anti-tubulin antibody and propidium iodide (to label the DNA) in metaphase stage of mitosis. Photographed by Dr. K. G. Murti, St. Jude Children's Research Hospital, Memphis, TN.

Technical content: Libby Yunger, Ph. D., Chloe McClanahan, B. Sc., Vicki Caligur, B. Sc.

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MISSION® TRC shRNA

MISSION® shRNA Human Tumor Suppressor Gene Panel

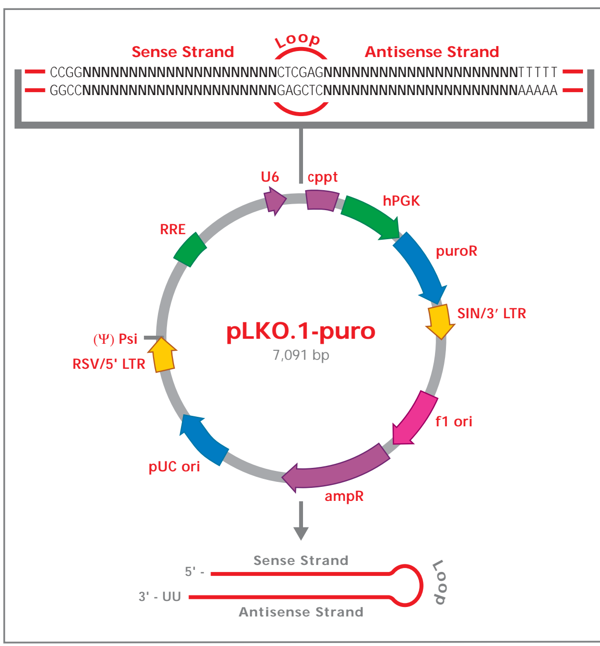
Silencing Tumor Suppressor Genes Using MISSION® TRC shRNA

RNAi methodologies have been utilized in small- to large-scale screening projects, allowing researchers to perform gene-silencing experiments in timeframes and target cells not previously possible. While siRNA screens have become fairly common, large-scale screening with shRNA is still evolving. Advantages of shRNA-based experiments include long-term knockdown and viral delivery to nontransfectable cell types.

The MISSION TRC shRNA Human Tumor Suppressor Gene Family Set is a panel of lentiviral-based shRNAs consisting of approximately 75 gene sets, each represented by 3–5 individual shRNA clones. The shRNA constructs included in the tumor suppressor set allow you to identify genes involved in cell survival by assisting in making a cell more sensitive, or more resistant, to an added chemotherapeutic agent through the knockdown of gene expression and resulting function.

Scientists at Sigma have utilized the MISSION TRC shRNA Human Tumor Suppressor Gene Family Set in order to address biologically relevant questions while simultaneously developing screening strategies that can be used by researchers in the field. The tumor suppressor gene family set delivers a potential pharmacogenomics solution, allowing you to profile the gene expression of a cancer cell line and compare it against the response of the cell line to chemotherapeutic treatment.[1]

In our study of paclitaxel resistance by the human lung cancer cell line, A549, various transcripts were down-regulated in the lung cancer cells using the tumor suppressor set. Several genes that are known to correlate with paclitaxel resistance, including *p53* and *VHL*, were identified by the panel. In addition, some genes, when down-regulated by these shRNAs, led to increased sensitivity of the cells to paclitaxel treatment.



Features of the pLKO.1-Puro vector allow for transient or stable transfection of the shRNA as well as production of lentiviral particles. Unlike adenovirus or murine-based MML or MSCV retroviral systems, lentiviral-based particles permit efficient infection and integration of the specific shRNA construct into differentiated and nondividing cells, overcoming low transfection and integration difficulties when using these cell lines. pLKO.1 allows for long-term knockdown and phenotypic observation and transduction of difficult or sensitive cell lines.

Features and Benefits

- Rapidly validate and compare results in multiple model systems – *spend more time analyzing your data and less time gathering it*
- Simplified, ready to transduce lentiviral format – *straightforward shRNA protocols let you progress to new cell line studies faster*
- shRNA enables you to down-regulate genes long-term – *easily transfer your experiments from in vitro to in vivo*

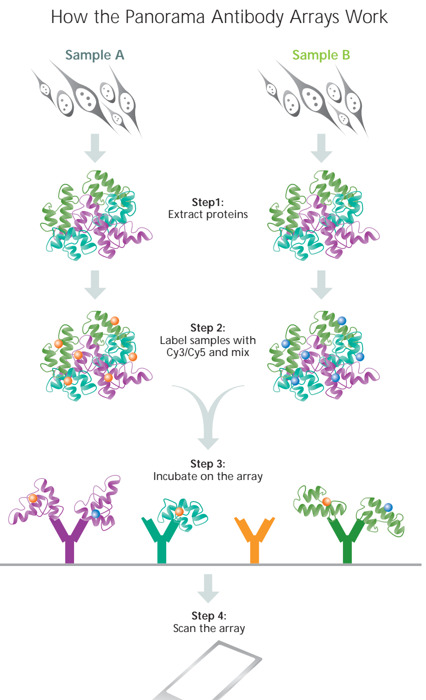
1. Ji, D., Deeds, S.L., and Weinstein, E.J., shRNAs Targeting Tumor Suppressor Genes, *Gen. Eng. News*, **27**, 26-27 (2007).

Ordering Information

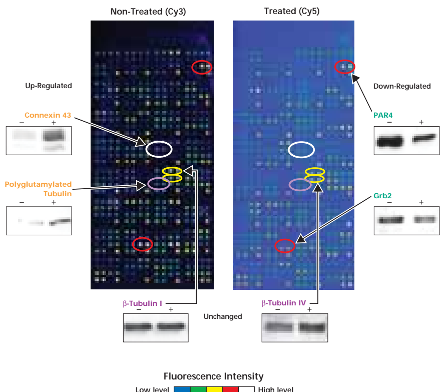
Cat. No.	Product Name	Pack Size
SH0531	MISSION TRC shRNA Human Tumor Suppressors	1 set
SHP001	MISSION Lentiviral Packaging Mix	0.25 mL 1.7 mL
H9268	Hexadimethrine bromide	5 g 10 g 50 g 100 g
P9620	Puromycin Ready Made Solution, 10 mg/mL in water	10 mL
N4888	Nutrient Mixture F-12 Ham with sodium bicarbonate, without L-glutamine, sterile-filtered liquid, cell culture tested	500 mL 6 × 500 mL 24 × 500 mL
QR0200	Quantitative RT-PCR ReadyMix™	1 kit
T7402	Paclitaxel from *Taxus brevifolia*	1 mg 5 mg 25 mg

For more information, please visit our Web site at **sigma.com/rnai.**

Panorama® Antibody Arrays



Induction with Retinoic Acid in Mouse F9 Cells



Using the Panorama Antibody Microarray – Cell Signaling Kit, proteins from mouse F9 cells are observed to be up-regulated or down-regulated in expected fashion following stimulation with retinoic acid. F9 cells were treated for 96 hours with all-trans-retinoic acid (10^{-7} M). Extracts were prepared from untreated and treated cells using Extraction/Labeling Buffer and labeled with Cy3 and Cy5, respectively. A mixture containing equal amounts of each labeled extract (5 µg/mL) was incubated on the array as described in the kit protocol. The same slide is shown at the two fluorescence emission wavelengths for Cy3 and Cy5. (Note that the blue background for Cy5 represents the normal and unavoidable interference of the nitrocellulose membrane.) Changes in expression level were confirmed by immunoblot, as indicated.

Protein Expression Profiling

Features

- Rapid, multiplex analysis of protein expression
- Profile 224 protein targets in half a day
- Antibodies react with human, mouse and rat species
- Comprehensive kits include buffers, extraction reagents, analysis software, and labware

High Throughput Protein Expression Analysis

Panorama Antibody Microarrays are designed to identify the changes in expression of multiple targets in a biological sample with a single assay. During cellular processes, mRNA undergoes a number of alternative processing steps prior to and following translation so there is often poor correlation between mRNA expression profiles and protein expression.[1] Antibody arrays provide an effective solution for analyzing biologically-relevant events at the proteome level.

Highly specific antibodies are arrayed in duplicate on nitrocellulose slides compatible with most DNA array scanners. Cy™3/Cy5-based detection chemistries may be used with cell or tissue extracts. Array antibodies are available separately for additional downstream analyses.

Applications

- Sample response to external treatments (e.g., starvation, addition of growth factors, serum, drugs/biomolecules)
- Comparison of different cell or tissue samples (clinical specimens, tissue, heart, brain, lung, etc.)
- Determine off-target effects in gene silencing (RNAi) studies

Components

Panorama Antibody Slides	QuadriPERM® Cell Culture Vessel
Extraction/Labeling Buffer	Protease Inhibitor Cocktail
Phosphatase Inhibitor Cocktail II	Benzonase® Ultrapure
Array Incubation Buffer	Collection Tubes, Polypropylene
Phosphate Buffered Saline, pH 7.4, with TWEEN® 20 (Washing Buffer)	SigmaSpin™ Post-Reaction Clean-Up Columns
Panorama Antibody List on diskette including analysis software	

1. Gygi, S.P., et al., *Mol. Cell Bio.*, **19**, 1720-1730 (1999)

Ordering Information

Cat. No.	Product Name	Pack Size
CSAA1	Panorama Antibody Microarray - Cell Signaling	1 kit
GRAA2	Panorama Antibody Microarray - Gene Regulations	1 kit
MPAA3	Panorama Antibody Microarray - MAPK & PKC Pathways	1 kit
PPAA4	Panorama Antibody Microarray - p53 Pathways	1 kit
XP725	Panorama Antibody Microarray - XPRESS Profiler725	1 kit

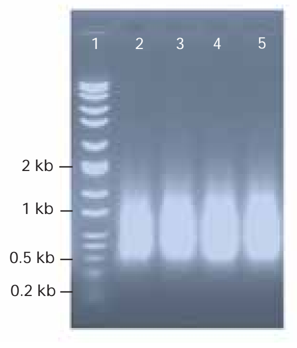
For more information, please visit our Web site at **sigma.com/arrays.**

GenomePlex® Single Cell Whole Genome Amplification Kit

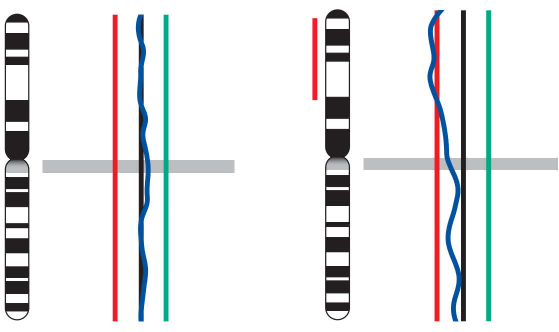
Whole Genome Amplification from a Single Cell

The GenomePlex Single Cell Whole Genome Amplification Kit **(Cat. No. WGA4)** is designed to amplify the genome of a single cell. This rapid and straightforward method provides million-fold amplification yielding microgram quantities of genomic DNA from a single cell. Traditional single cell whole genome amplification methods yield insufficient quantities with significantly biased representation. In contrast, the GenomePlex technology provides enhanced amplification efficiency by using WGA DNA polymerase.

The Single Cell WGA kit includes all of the reagents necessary for cell lysis and subsequent whole genome amplification. Single cells can be isolated by fluorescence-activated cell sorting (FACS), laser capture microdissection (LCM), dilution, or any other applicable method. Single Cell WGA has been successfully applied to Comparative Genome Hybridization (CGH), STR analysis of amniocentesis samples, and genomic analysis of *in vitro* fertilized embryos.



Human leukemia U937 cells were isolated using flow cytometric analysis and sorting (FACS), lysed, and amplified using the GenomePlex Single Cell Whole Genome Amplification Kit. The DNA was then purified with the GenElute™ PCR Cleanup Kit. An estimated million-fold amplification from the WGA process resulted in a final yield ranging from 5.4-6.2 µg. The Single Cell WGA Kit produces consistent yield and size range as visualized by a 1% agarose gel.



DNA from normal and tumorigenic human kidney cells were amplified using the GenomePlex Single Cell WGA Kit. Amplified material was hybridized to metaphase BAC arrays to determine chromosomal karyotype. Data that falls outside of the red line indicates chromsomal loss, while data that continues past the green line suggests chromosomal amplification. As expected the control sample demonstrated a balanced chromosomal copy number. Chromosome 3 for the amplified kidney tumor single cell displayed under representation as depicted by the red bar. These results match previous microarray work using an amplification method that took three days. GenomePlex technology accurately amplifies genomic material down to single cell resolution. Data is courtesy of Dr. Michael Speicher from the Institute of Human Genetics, TU Munich.

Ordering Information

Cat. No.	Product Name	Pack Size
WGA2	GenomePlex Complete Whole Genome Amplification (WGA) Kit	10 reactions 50 reactions
WGA4	GenomePlex Single Cell Whole Genome Amplification Kit	10 reactions 50 reactions
WGA3	GenomePlex WGA Reamplification Kit	50 reactions
WGA5	GenomePlex Tissue Whole Genome Amplification Kit	10 reactions 50 reactions
NA1020	GenElute PCR Clean-Up Kit	1 kit
G1N10	GenElute Mammalian Genomic DNA Miniprep Kit	1 kit
S4438	SYBR® Green JumpStart™ Taq ReadyMix™	100 reactions 500 reactions

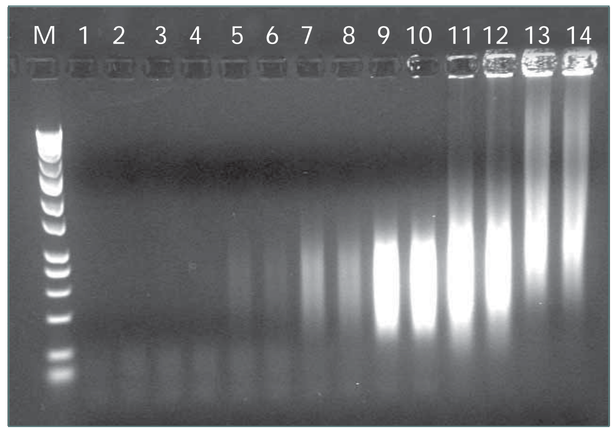
To see Sigma's complete line of Whole Genome Amplification products, visit our Web site at **sigma.com/wga.**

GenomePlex® Complete Whole Genome Amplification Kit

Amplification of Genome-Representative DNA from Limited Starting Material

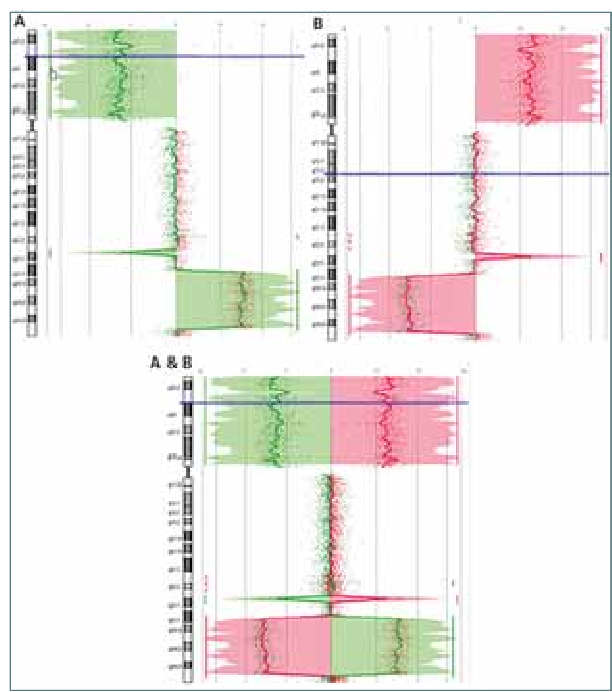
The GenomePlex Complete Whole Genome Amplification Kit (**Cat. No. WGA2**) contains everything required for whole genome amplification including an optimized enzyme, WGA DNA Polymerase. The WGA DNA Polymerase provides increased accuracy in amplification, as evidenced by producing no amplicon in the negative control reactions. WGA has been used in a variety of applications, and is suitable for use with purified genomic DNA from a variety of sources including blood cards, whole blood, buccal swabs, tissue, soil, plant, and serum. GenomePlex WGA uses nanogram quantities of starting genomic DNA, which after PCR yields on average 10 µg of amplified DNA. After purification, the WGA product can be analyzed in a manner similar to any genomic or chromosomal DNA sample. A number of downstream applications may be performed including TaqMan® assays, CGH analysis, SNP analysis, and sequencing.

Achieve Robust Amplification Representative of the Original Input Genome



WGA was performed on increasing concentrations of human genomic DNA. Amplification product can be detected on an agarose gel with as little as 10 pg of input DNA. Optimal performance is found with 1 to 10 ng of starting material. Increasing the amount of input DNA to 100 ng is not recommended.

Lane M: DNA Marker
Lanes 1,2: no template Lanes
Lanes 3,4: 1 pg DNA Lane
Lanes 5,6: 10 pg DNA Lanes
Lanes 7,8: 100 pg DNA
Lanes 9,10: 1 ng DNA
Lanes 11,12: 10 ng DNA
Lanes 13,14: 100 ng DNA

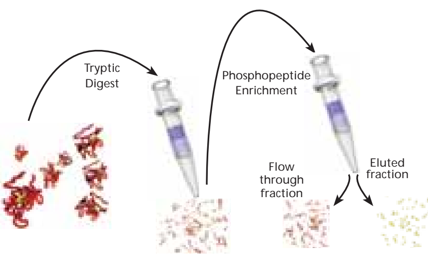


GenomePlex WGA was performed on genomic DNA isolated from HT29 colon carcinoma cells and from a healthy human male. 2.5 µg of WGA product was labeled with Cy™3 or Cy5 dye using the Genomic DNA Labeling Kit PLUS (Agilent). The entire labeled sample was loaded onto an Agilent Human Genome CGH Microarray 105A. Specific activities were between 28 and 43 for all samples, and always within 50% of samples being compared. The dye swaps **(A & B)** demonstrate that there was no bias in the DNA labeling and the aberrations detected are consistent with the HT-29 karyotype.

Looking for a high-throughput system for the rapid and highly representative amplification of genomic DNA from trace amounts of starting material? Visit **sigma.com/wgaautomation** for automated WGA protocols and methods.

NEW! Technical note on Agilent Array CGH with WGA, visit **sigma.com/wgacgh.**

PhosphoProfile™ Phosphopeptide Enrichment Kit



Workflow highlighting the alternate use of trypsin spin columns **(Cat. No. TT0010)** for digestion followed by selective enrichment of phosphopeptides from sample.

The Complete Solution Kit
24 samples up to 25 nmoles phosphopeptide each.

Features and Benefits

- Mass spec compatible – *save time, reduce sample handling and potential loss*
- Proteomics Grade Trypsin – *for clean and complete digests*
- Phos-Select™ Gallium Spin Columns – *high capacity, novel Ga+3 silica media for fast, unbiased capture and recovery*
- Controls – *validate your process for confidence and reporting*
- Buffers – *enzyme reaction, binding, washing and elution formulations optimized for robust performance*
- Consumable equipment included – *matched equipment means no risk of sample loss, additional purchases, or waste*

Phosphoproteomics — Phosphopeptide Enrichment

Matrix Assisted Laser Desorption/Ionization, Time of Flight (MALDI-TOF) or Electrospray Ionization (ESI) Mass Spectrometry of phosphopeptides from tryptic protein digests are powerful tools for characterization and identification of phosphorylation sites. A combination of low intrinsic abundance, inefficient ionization, and/or signal suppression of most phosphopeptides may limit or even prevent detection, unless the phosphopeptide content is significantly enriched prior to analysis. This kit conveniently includes all materials needed to enrich phosphopeptides from digested samples in a robust and unbiased manner (see binding comparison). The phosphopeptide capture matrix is a novel Ga^{+3} chelate silica based on a proprietary nitriloacetic acid (NTA) analog. The silica beads are approximately 20 microns in diameter with a pore size of 1,000 Angstroms. The matrix is packed into spin columns for easy, microscale affinity capture of phosphopeptides.

Binding Comparison: Demonstrating enrichment of phosphopeptides for IMAC technologies.

Standard phosphopeptides representing the three most common sites of phosphorylation (phosphoserine, phosphothreonine, and phosphotyrosine) were used. The lyophilized solids were first dissolved in water, and an approximately equimolar mixture of the peptides was formulated. Each phosphopeptide was added to a BSA digest at a weight ratio of ~1.7% to produce a total phosphopeptide content of ~5% by weight. Quantitation results are given in the table below. Note that competitor A, B, and C technologies were biased in selecting Peptide 2, while Sigma's technology bound and eluted the peptides in approximately the same ratio as applied to the column.

Kit	Recovery of phosphopeptide standards				Specificity*
	1	2	3	Total	
Sigma	59%	52%	74%	59%	50%
Competitor A	6%	19%	11%	13%	28%
Competitor B	39%	56%	17%	42%	28%
Competitor C	37%	65%	37%	46%	25%

Performance summary of the IMAC technologies tested within this study.

*Specificity was measured as a percentage of the total HPLC peak area corresponding to phosphorylated peptides that appeared in the elution fractions.

Ordering Information

Cat. No.	Product Name	Pack Size
PP0410	PhosphoProfile I Phosphopeptide Enrichment Kit	1 kit
TT0010	Trypsin Spin Columns Proteomics Grade	10 each

For more information, please visit our Web site at **sigma.com/pep.**

ABC Transporters and Cancer Drug Resistance

Role of ABC Transporters and Multi-Drug Resistance Reversal Using RNA Interference (RNAi)*

Chemotherapy is the treatment of choice against many types of cancer. However, over time chemotherapeutic drugs can become less effective due to the development of resistance that involves a group of membrane proteins. These multi-drug transporters expel cytotoxic molecules from the cell, thus keeping intracellular drug concentrations below the cell-killing threshold. These transporters belong to the superfamily of ATP Binding Cassette (ABC) proteins that are present in all organisms from bacteria to humans. The transport activity of ABC proteins has an important effect on the efficacy of pharmaceuticals by modulating the absorption, distribution, and excretion of these xenobiotics.

ABC transporter proteins are located in the plasma membrane of cells and in the membranes of cellular organelles where they mediate the transport of various substrate molecules. These substrates exhibit a wide variety of chemical structures. Most ABC proteins are active transporters, which utilize the energy generated by ATP hydrolysis; however, some ABC transporters form transmembrane channels.

Cancer Multi-Drug Resistance - The Players

Numerous clinical data revealed that the multi-drug resistance phenotype in tumors is associated with the overexpression of certain ABC transporters, termed multi-drug resistance (MDR) proteins. P-glycoprotein (P-gp, MDR1, ABCB1) was the first discovered ABC transporter[1-3] and is likely to be responsible for the most widely observed mechanism in clinical multi-drug resistance.[4-7] Soon after the cloning and characterization of MDR1, it became evident that other efflux pumps also play significant roles in transport-associated drug resistance. Two other ABC transporters have definitively demonstrated participation in the multi-drug resistance of tumors: the multi-drug resistance protein 1 (MRP1, ABCC1), and the mitoxantrone resistance protein (MXR/BCRP, ABCG2).[7-11]

Basic Mechanism of Cancer Multi-Drug Resistance and Substrate Specificity of MDR-ABC Transporters

The generally accepted mechanism of multi-drug resistance is that the MDR proteins actively expel the cytotoxic drugs from cells, maintaining the drug concentration within the cells below the toxic level. The drug efflux mediated by these primary active transporters is driven by the energy of ATP hydrolysis. Tumors with MDR protein overexpression (e.g., hepatomas, lung or colon carcinomas) often show primary (or intrinsic) resistance to chemotherapy treatment. In addition, chemotherapy itself may induce the overexpression of these proteins, resulting in the multi-drug resistant clones becoming less sensitive to treatment (secondary drug resistance).[12-14]

The most intriguing characteristic distinguishing the MDR proteins from other mammalian transporters is their broad substrate specificity. Unlike other selective (classical) transport proteins, multi-drug transporters recognize and handle a large number of structurally diverse, mainly hydrophobic compounds, which explains cross-resistance to several chemically unrelated compounds, a characteristic feature of the multi-drug resistance phenotype.[4-7] In addition to their overlapping substrate specificity, each transporter can handle unique compounds. The following table of MDR-substrate anticancer agents provides a selection of anticancer agents available from Sigma and identifies the key ABC transporter(s) responsible for each agent's cellular efflux.

Circumvention of Cancer Multi-Drug Resistance

Prevention of clinical multi-drug resistance should significantly improve therapeutic response in a large number of cancer patients. The initial search for pharmacological modulators of MDR transporters yielded two generations of compounds having poor clinical response profiles. Therefore, there has been a shift to structure-based drug design to synthesize modulator compounds characterized by a high affinity to MDR transporters.[15,16] Additionally, research that utilizes siRNA and shRNA-mediated RNAi-based gene silencing methodology has recently delivered promising results.

MDR Modifying Agents

MDR modifying agents, which competitively or non-competitively inhibit the MDR proteins, may increase the cytotoxic action of multi-drug resistant related drugs by preventing the active efflux of these drugs from the target cells. The co-application of an "MDR-modulating" compound in combination with chemotherapy would be expected to significantly improve the cancer cure rate. The first-generation modulators consisted of compounds that were already in clinical use. Calcium channel blockers, quinine derivatives, calmodulin inhibitors, and the immunosuppressive agent cyclosporin A, were all shown to interact with the MDR transporters *in vitro* and *in vivo*. These modulators were not specifically developed for MDR protein inhibition, and their inherent low affinity for MDR transporters resulted in a high toxicity profile and were never shown to inhibit P-gp in patients.[15-18]

Most of the second-generation modulators were derivatives of the first-generation compounds that retained MDR modulatory effects, but with reduced activity toward other physiological targets. Prominent examples of this group are R-verapamil, biricodar (VX-710), and valspodar (PSC-833). These modulators were shown to inhibit P-gp in patients, but further study revealed significant pharmokinetic interaction with several anticancer drugs, which delayed excretion of the anticancer agent, resulting in toxicity requiring reduction of anticancer drug doses.[15-18]

The third-generation MDR modulators are designed to interact with specific MDR transporters[7, 11-13, 19, 20] with high affinity and with efficacy at nanomolar concentrations. Development of this class of MDR modulators employed combinatorial chemistry to produce potent and selective inhibitors. Examples are the small hydrophobic peptide derivatives named reversins, which were shown to have a strong inhibitory effect on P-gp/MDR1-mediated drug efflux without any toxic effect in the control cells.[21] Ongoing clinical trials using third-generation MDR modulators for specific cancer types include: elacridar (GF120918), tariquidar (XR9576), zosuquidar (LY335979), laniquidar (R101933), and ONT-093. Still the shortcomings of earlier generation modulators continue to exist.[17, 22] Other approaches to prevent the expression or function of multi-drug transporters are being considered, including the use of MDR protein targeted antibodies, the use of carriers that deliver these drugs selectively to tumor tissues, and the use of RNA interference.

SIGMA

ABC Transporters and Cancer Drug Resistance

Multi-Drug Resistance Reversal Using RNAi

The stable reversal of MDR protein-mediated drug efflux by RNAi technology has been demonstrated *in vitro* for MDR1, MXR, MRP2, and MDR3. One of the early multi-drug resistance studies using RNAi technology reported a complete suppression of MDR1 expression on the mRNA and protein level in human gastric carcinoma cells.[23] A subsequent study further demonstrated inhibition of both MDR1 and MDR3 expression in conjunction with the reversal of paclitaxel resistance in human ovarian cancer cells. Treatment of ovarian cancer cell lines with either chemically synthesized siRNAs or transfection with specific vectors that express targeted siRNAs resulted in decreased mRNA and protein levels. In this study, MTT assays of siRNA-treated cells demonstrated 7 to 12.4-fold reduction of paclitaxel resistance in the lines treated with the synthesized siRNA of MDR1 and 4.7 to 7.3-fold reduction of paclitaxel resistance in the cell lines transfected with siRNA of MDR1 expressing vectors.[24] A more recent study surprisingly showed that the MDR1 phenotype in human hepatoma cells was completely reversed by using two transfected clones.[25] Aside from the more frequently studied MDR1 phenotype, reversal of the drug-resistant MXR and MRP2 phenotype using both siRNA and shRNA-mediated approaches was also demonstrated in human carcinoma cells.[26, 27]

In a pre-clinical study the ablation of MDR1 in cells stably transduced with shRNA was functionally confirmed by increased sensitivity of MDR1-transfected cells toward the cytotoxic drugs vincristine, paclitaxel, and doxorubicin as well as by transport of ^{99m}Tc-sestamibi. In the same study, shRNA-mediated down-regulation of MDR activity in tumor implants in living animals was followed by direct noninvasive bioluminescence imaging using the fluorophore coelenterazine, a known MDR1 transport substrate. Additionally, a MDR1-firefly luciferase (MDR1-FLuc) fusion construct was used to document the effect of shRNA delivered *in vivo* on MDR1-FLuc protein levels with D-luciferin bioluminescence imaging.[28] A similar study validated selective MRP2 gene function inhibition: after the intravenous delivery of siRNA effectors into mice, researchers observed a significantly reduced calcein excretion rate and resultant siRNA accumulation in the kidney.[29]

RNAi is proving to be a powerful laboratory tool for better understanding the multi-drug resistance genotype and phenotype. Its future therapeutic utility in suppressing gene expression in cancer patients will likely be dependent on the availability of effective RNAi delivery systems. Lessons can be learned from the history of gene therapy and antisense technologies. These technologies ultimately failed to produce successful clinical outcomes due to potentially harmful and inefficient delivery systems. The use of RNAi in complex genetic diseases, such as cancer, will not see a quick and straightforward transition from research to clinical success, but with time the promise of viable RNAi therapies may be realized.[30] Additionally, innovative technologies combined with new directions in the study of ABC transporters will lead to an understanding of whether or not ABC transporters are important molecular targets for anticancer drug development.

*Technical content provided by: Balázs Sarkadi[1], Gergely Szakács[1,2] and András Váradi[3]
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2. National Cancer Institute, NIH, Bethesda, MD, USA
3. Institute of Enzymology, Hungarian Academy of Sciences, Budapest, Hungary

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For more information on siRNA available through Sigma-Genosys and the comprehensive MISSION TRC shRNA library, targeting 15,000 human and 15,000 mouse genes, visit our Web site at sigma.com/rnai.

To obtain more information on specific ABC transporter shRNA clones, email RNAi@sial.com.

MDR-substrate Anticancer Agents available from Sigma

Compound	ABC Transporter(s)[1]	Cat. No.	Description	Pack Size
Actinomycin D	MDR1	**A1410**	from *Streptomyces* sp., ~98% (HPLC)	2 mg 5 mg 10 mg 25 mg 100 mg
		A4262	from *Streptomyces* sp., ~95% (HPLC)	2 mg 5 mg 10 mg 25 mg
Carmustine	MRP1	**C0400**	≥98%	25 mg 100 mg
Chlorambucil	MRP1	**C0253**		1 g 10 g
Colchicine	MDR1	**C9754**	~95% (HPLC)	100 mg 500 mg 1 g 5g
Cyclophosphamide monohydrate	MRP1	**C0768**	Bulk package, 97.0-103.0% (HPLC)	100 mg 500 mg 1 g 5 g
		C7397	ISOPAC®[2]	1 g
Daunorubicin hydrochloride	MDR1 MRP1 MXR	**D8809**	Meets USP testing specifications	1 mg 10 mg 50 mg
***cis*-Diammineplatinum(II) dichloride (cisplatin)**	MRP1	**P4394**		25 mg 100 mg 250 mg 1 g
Doxorubicin HCl	MDR1, MRP1, MXR	**D1515**	~98% (TLC)	10 mg
Etoposide	MDR1 MRP1	**E1383**	Synthetic, ≥98%	25 mg 100 mg 250 mg
Folinic acid calcium salt (Leucovorin)	MRP1	**F7878**	≥90%	100 mg 500 mg 1 g
Hydroxyurea	MRP1	**H8627**	≥98% (TLC)	1 g 5 g 10 g 25 g 100 g
Methotrexate hydrate	MRP1	**A6770**	>98% (HPLC)	10 mg 25 mg 100 mg 500 mg 1 g
Mitomycin C	MDR1	**M0503**	from *Streptomyces caespitosus* Vial contains 2 mg mitomycin C and 48 mg NaCl.	2 mg 5 X 2 mg 10 X 2 mg 50 X 2 mg
Mitoxantrone dihydrochloride	MDR1 MXR	**M6545**	≥97% (HPLC)	10 mg 50 mg
Paclitaxel	MDR1	**T7402**	from *Taxus brevifolia*, ≥95% (HPLC)	1 mg 5 mg 25 mg
Tamoxifen	MDR1 MRP1	**T5648**	≥99%	1 g 5 g
Vinblastine sulfate salt	MDR1 MRP1	**V1377**	≥97% (TLC)	1 mg 5 mg 10 mg 25 mg 50 mg
Vincristine sulfate salt	MDR1 MRP1	**V8879**	95.0-105.0% (HPLC)	1 mg 5 mg 25 mg

1. The ABC transporters indicated are the proteins which are responsible for the cellular efflux of the specific anticancer agent.
2. ISOPAC® products are packaged in 100 mL serum bottles with a butyl rubber stopper and aluminum tear seal. Injecting any compatible solvent permits preparation of any desired strength solution without exposure.

ABC Transporters and Cancer Drug Resistance

ABC Transporter Membrane Proteins

MDR1 human

ABCB1; Pgp

The MDR1 protein is involved in cancer drug resistance and in the transport of hydrophobic drugs and xenobiotics in the bowel, kidney, liver, and the blood-brain barrier. Drugs interacting with this protein may be useful for the reversal of cancer drug resistance or increasing the absorption or brain entry of various pharmacological agents.

Membrane preparation, for ATPase, recombinant, expressed in Sf9 cells

Supplied as isolated Sf9 cell membranes containing human MDR1 (Pgp) suspended in TMEP solution.

Distributed for SOLVO Biotechnology, Inc.

-70°C DRY ICE

M9194	500 μL

Mdr1b from rat

The MDR1 protein is involved in cancer drug resistance and in the transport of hydrophobic drugs and xenobiotics in the bowel, kidney, liver, and the blood-brain barrier. In rodents, there are two MDR1 genes, mdr1a and mdr1b, while in human, there is a single MDR1 gene. Based on function and tissue distribution in rodents, the equivalent of the human MDR1 gene product (PgP) is the product of the rodent mdr1b gene. There have been no reported significant differences in function, substrate specificity, or substrate affinity between these two proteins.

Membrane preparation, for ATPase, recombinant, expressed in Sf9 cells.

Supplied as isolated Sf9 cell membranes containing rat Mdr1b suspended in TMEP solution.

Distributed for SOLVO Biotechnology, Inc.

-70°C DRY ICE

M9319	500 μL

MRP2 human

MRP2 (ABCC2) is an organic anion transporter found in the liver, kidney, and gut epithelium apical membranes. The transport of glucuronate conjugates plays a role in the detoxification of endogenous and xenobiotic substances, and may cause multidrug resistance (MDR) in tumor cells.

Membrane preparation, for Vesicular Transport, recombinant, expressed in Sf9 cells

Supplied as isolated Sf9 cell membranes containing human MRP2 suspended in TMEP solution.

Distributed for SOLVO Biotechnology, Inc.

-70°C DRY ICE

M9069	500 μL

Mrp2 from rat

MRP2 (ABCC2) is an organic anion transporter found in liver, kidney, and gut epithelium apical membranes. The transport of glucuronate conjugates plays a role in the detoxification of endogenous and xenobiotic substances, and may cause multidrug resistance (MDR) in tumor cells. The rat Mrp2 transporter shows 72.3% sequence identity and 85.6% sequence similarity with human MRP2. Both transporters are expressed on the canalicular membrane of the liver and are known to be responsible for the transport of some organic molecules and their conjugates to the bile.

Membrane preparation, for ATPase, recombinant, expressed in Sf9 cells

Supplied as isolated Sf9 cell membranes containing rat Mpr2 suspended in TMEP solution.

Distributed for SOLVO Biotechnology, Inc.

-70°C DRY ICE

M9694	500 μL

MXR human

MXR membrane vesicles are purified from recombinant baculovirus transduced Sf9 cells or selected, MXR over-expressing mammalian cells.

Distributed for SOLVO Biotechnology, Inc.

Membrane preparation, wild type variant, for Vesicular Transport

Supplied as isolated mammalian cell membranes containing human MXR (wild type variant) suspended in TMEP solution.

The MXR transporter can be produced in sufficient quantity by selected, MXR over-expressing mammalian cell lines.

-70°C DRY ICE

M9569	500 μL

Membrane preparation, wild type variant, for Vesicular Transport, recombinant, expressed in Sf9 cells

Supplied as isolated Sf9 cell membranes containing wild type human MXR suspended in TMEP solution.

The MXR transporter can be expressed in Sf9 insect cells using the baculoviral expression system, yielding high protein levels (up to 5% of total membrane protein) in the cell membrane of infected cells.

-70°C DRY ICE

M9444	500 μL

MXR Control

Membrane preparation, from Sf9 cells

Control for ATPase and vesicular transport assays.

Supplied as isolated Sf9 cell membranes containing a defective MXR gene suspended in TMEP solution.

Distributed for SOLVO Biotechnology, Inc.

-70°C DRY ICE

M9944	500 μL

MXR Control

Membrane preparation, mammalian

Control for ATPase and vesicular transport assays.

Supplied as isolated mammalian cell membranes (not selected for transport expression) suspended in TMEP solution.

Distributed for SOLVO Biotechnology, Inc.

-70°C DRY ICE

C3992	500 μL

Antibodies to ABC Transporters

Monoclonal Anti-Breast Cancer Resistance Protein antibody produced in mouse

Anti-ABCG2; Anti-BCRP

250 µg/mL, clone BXP-21, tissue culture supernatant
Immunogen: fusion protein containing human BCRP (amino acids 271-396) and maltose-binding protein. Reacts with an internal epitope of BCRP. Does not cross-react with the human MDR1, MRP1, MRP2 gene products.

Supplied in serum-free medium containing 0.7% bovine serum albumin and 0.1% sodium azide.

Species reactivity: human

Antigen mol wt ~70 kDa

Application(s)
Immunocytochemistry............................1:20-1:50 using acetone-fixed cytospin preparations
Immunohistochemistry (frozen sections)......... 1:20 using acetone-fixed frozen sections
Immunohistochemistry (formalin-fixed, paraffin-embedded sections)
....suitable using pretreated human tissue
Immunoblotting ....suitable
Isotype...IgG2a

-20°C DRY ICE

B7059	1 mL

Anti-Breast Cancer Resistance Protein antibody produced in rabbit

Anti-BCRP

Affinity isolated antibody
Immunogen: synthetic peptide corresponding to amino acid residues 150-167 of human breast cancer resistance protein with C-terminal added cysteine, conjugated to KLH.

Solution in 0.01 M phosphate buffered saline containing 15 mM sodium azide.

Species reactivity: human, mouse

Antigen mol wt ~70 kDa

Application(s)
Immunoblotting.............................2.5-5 µg/mL using whole extract of human term placenta or mouse kidney and a chemiluminescent detection reagent
Immunohistochemistry (formalin-fixed, paraffin-embedded sections) .... 20-40 µg/mL using heat-retrieved tissue sections from human term placenta by indirect immunoperoxidase staining of syncytiotrophoblasts.

-20°C DRY ICE

B7185	200 µL

Monoclonal Anti-P-Glycoprotein (MDR) antibody produced in mouse

Clone F4, ascites fluid
Immunogen: mixture of human and hamster drug-resistant whole cells and crude plasma membranes.

The antibody recognizes an epitope located in the amino terminal half of P-glycoprotein (Pgp), at the third extracellular loop of the molecule. The epitope is resistant to formalin fixation and periodate oxidation.[1] The antibody detects specifically human MDR1 P-glycoprotein, but does not appear to recognize the human MDR3 product[1], nor the mouse mdr1a, mdr1b or the mdr3 P-glycoprotein.[2]

Contains 15 mM sodium azide

Species reactivity: human, hamster

Antigen mol wt 170-180 kDa

Application(s)
Immunoblotting...suitable
Radioimmunoassay .... suitable using cell-surface RIA
Immunoprecipitation .... suitable
Immunohistochemistry (frozen sections) ....suitable
Immunocytochemistry .... suitable
Flow cytometry ....suitable
Indirect ELISA ....suitable
Immunohistochemistry (formalin-fixed, paraffin-embedded sections)
....1:500 using human kidney sections
Isotype IgG1

Lit. cited: 1. Chu, T.M., et al., *Hybridoma* **12**, 417 (1993)
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-20°C DRY ICE

P7965	0.2 mL

Monoclonal Anti-MDR3 P-Glycoprotein antibody produced in mouse

250 µg/mL, clone P_3II-26, tissue culture supernatant
Immunogen: MDR3 P-gp (amino acids 629-692) GST fusion protein.

Reacts with an internal epitope of MDR3. Does not cross-react with human MDR1 P-gp.

Supplied in serum-free medium containing 0.7% bovine serum albumin and 0.1% sodium azide.

Species reactivity: human

Application(s)
Immunocytochemistry............................1:20-1:50 using acetone-fixed cytospin preparations
Immunohistochemistry (frozen sections).............................. 1:20 using acetone-fixed sections
Immunohistochemistry (formalin-fixed, paraffin-embedded sections)
....Not suitable
Immunoblotting...suitable
Isotype..IgG2b

-20°C DRY ICE

M7317	1 mL

SIGMA

ABC Transporters and Cancer Drug Resistance

Monoclonal Anti-MRP1 antibody produced in mouse

Anti-Multidrug Resistance Associated Protein 1

~2 mg/mL, clone QCRL-1, purified immunoglobulin

Immunogen: non-denatured membrane preparations of H69AR human small cell lung cancer cell line, which highly expresses MRP1. The epitope resides within amino acids 918-924 of human MRP1. Does not cross-react with the human MDR1 and MDR3, mouse MRP1, and human MRP2, MRP3, MRP4, MRP5, and MRP6 gene products. The antibody may cross-react with canine MRP1.

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide.

Species reactivity: human

Antigen mol wt ~190 kDa

Application(s)

Flow cytometry .... 0.5-2 µg/mL using human H69AR cells
Immunoblotting .... suitable
Immunoprecipitation .... suitable
Immunohistochemistry .... suitable
Immunocytochemistry .... suitable
Isotype IgG1

-20°C DRY ICE

M9067	200 µL

Anti-MRP2 antibody produced in rabbit

Anti-ABCC2; Anti-cMOAT; Anti-cMRP; Anti-Multidrug Resistance Associated Protein 2

Affinity isolated antibody

Immunogen: synthetic C-terminal peptide corresponding to amino acids 1528-1545 of human MRP2 conjugated to KLH.

Additional lower bands including an approx. 175 kDa band representing an immature unglycosylated form may be detected in various extract preparations.

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Species reactivity: human, rat

Antigen mol wt ~190 kDa

Application(s)

Immunoblotting .... 1:1,000 using whole extract of HepG2 human hepatoblastoma cells
Immunoblotting .... 1:1,000 using 293T cells expressing recombinant human MRP2
Indirect immunofluorescence .... 1:100 using paraformaldehyde-fixed HepG2 cells
Indirect immunofluorescence .... 1:100 using rat liver frozen sections

-20°C DRY ICE

M8316	200 µL

Monoclonal Anti-MRP2 antibody produced in mouse

Anti-ABCC2; Anti-cMOAT; Anti-cMRP; Anti-Multidrug Resistance Associated Protein 2

~1.5 mg/mL, clone CPR96, purified immunoglobulin

Immunogen: synthetic C-terminal peptide corresponding to amino acid residues 1528-1545 of human MRP2 with N-terminal added cysteine conjugated to KLH.

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Species reactivity: human

Antigen mol wt ~180 kDa

Application(s)

Immunoblotting .... 1-2 µg/mL using cell extracts of 293T cells transfected with human MRP2
Indirect ELISA .... suitable
Array .... suitable
Isotype IgG1

-20°C DRY ICE

M3692	200 µL

Anti-MRP3 antibody produced in rabbit

Anti-Multidrug Resistance Associated Protein 3

Affinity isolated antibody

Immunogen: synthetic C-terminal peptide of human MRP3 (amino acids 1507-1527) with N-terminal cysteine conjugated to KLH. The sequence in mouse and rat differs by three residues.

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Species reactivity: human, rat

Antigen mol wt ~180 kDa

Application(s)

Immunoblotting .... 2.5-5 µg/mL using whole extract of cultured human colon carcinoma HCT-116 cells
Indirect immunofluorescence .... 15-30 µg/mL using frozen sections of rat skin (outer root sheaths of hair follicles)

-20°C DRY ICE

M0318	200 µL

Monoclonal Anti-MRP5 antibody produced in rat

Anti-Multidrug Resistance Associated Protein 5

250 µg/mL, clone M$_5$I-1, tissue culture supernatant

Immunogen: human MRP5 (amino acids 82-168) bacterial fusion protein. Reacts with an internal epitope of MRP5. Does not cross-react with the human MDR1, MRP1, MRP2, MRP3 gene products.

Supplied in serum-free culture medium containing 0.7% bovine serum albumin and 0.1% sodium azide.

Species reactivity: human

Antigen mol wt 190-200 kDa

Application(s)

Immunocytochemistry .... 1:20-1:50 using acetone-fixed cytospin preparations
Immunohistochemistry (frozen sections) .... 1:20 using acetone-fixed frozen sections
Immunoblotting 1:20-1:50
Isotype IgG2a

-20°C DRY ICE

M6067	1 mL

Compounds for MDR Detection

bisBenzimide H 33342 trihydrochloride

bisBenzimide; 2′-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole trihydrochloride; HOE 33342; Hoechst 33342
$C_{27}H_{28}N_6O \cdot 3HCl \cdot xH_2O$ FW 561.93 (Anh) [23491-52-3]

Membrane-permeable, fluorescent DNA stain with low cytotoxicity that intercalates in A-T regions of DNA.

Useful for staining DNA, chromosomes and nuclei. May be used for fluorescence microscopy or flow cytometry.

Excitation max. = 346 nm

Emission max. = 460 nm

≥95% (TLC)

-20°C EC No. 2456911 BRN 1234011

B2261	25 mg, 100 mg, 500 mg, 1 g

Calcein

Bis[*N,N*–bis(carboxymethyl)aminomethyl]fluorescein; Fluorescein-bis(methyliminodiacetic acid); Fluorexon

$C_{30}H_{26}N_2O_{13}$ FW 622.53 [1461-15-0]

Used for the fluorometric determination of calcium and EDTA titration of calcium in the presence of magnesium.

-20°C EC No. 2159571 BRN 8181068

C0875	5 g, 10 g, 25 g

Calcein AM solution

$C_{46}H_{46}N_2O_{23}$ FW 994.86 [148504-34-1]

≥4 mM in DMSO, ≥90% (TLC)
Fluorescent cell permeable derivative of calcein.

-20°C

C1359	100 µL

Coelenterazine, native

CLZN; 3,2-Dihydro-2-(p-hydroxybenzyl)-6-(p-hydroxyphenyl)-8-benzylimidazo-lo[1,2-a]pyrazin-3-one
$C_{26}H_{21}N_3O_3$ FW 423.46 [55779-48-1]

Luminophore of the aequorin complex which is oxidized by oxygen to illuminate at 465 nm when Ca^{2+} binds to the complex; used to measure Ca^{2+} concentration in cells with high sensitivity and large dynamic range.

-20°C

C2230	50 µg

Luciferase from *Photinus pyralis* (firefly)

[61970-00-1] E.C. 1.13.12.7

Lyophilized powder, 15-30 × 10^6 light units/mg protein
Arsenate free.

Lyophilized powder approximately 20% protein; balance is primarily NaCl, HEPES buffer salts, and carbohydrate.

Mol wt 120 kDa (two non-identical subunits, each containing four free thiol groups, one of which is necessary for activity)

Composition Protein ~20% ($E^{1\%}_{280}$)

Chromatographically prepared and crystallized.

Sold on the basis of protein content

One light unit produces a biometer peak height equivalent to 0.02 µCi of ^{14}C in PPO/POPOP cocktail. Light units measured in 50 µl assay mixture containing 5 pmol ATP and 7.5 nmol luciferin in Tris-glycine buffer, pH 7.6, at 25 °C.

Sensitivity ≤1 femtomole ATP (using 0.2 µg of luciferase and suitably sensitive liquid scintillation counters or luminometers)
ATPase <5 nanomolar units/mg protein
Nucleoside diphosphokinase <20 nanomolar units/mg protein

-20°C EC No. EINECS

L9506	1 mg, 2 mg, 10 mg

D-Luciferin

4,5-Dihydro-2-(6-hydroxy-2-benzothiazolyl)-4-thiazolecarboxylic acid; Firefly luciferin; (S)-2-(6-Hydroxy-2-benzothiazolyl)-2-thiazoline-4-carboxylic acid
$C_{11}H_8N_2O_3S_2$ FW 280.32 [2591-17-5] EC No. 2199813 BRN 30484

Synthetic, SigmaUltra, ≥99% (HPLC)
Highly purified grade with reduced levels of inhibitor relative to L9504

L6152	1 mg, 10 mg

Synthetic

L9504	1 mg, 5 mg, 10 mg, 50 mg, 100 mg

Rhodamine 123

2-(6-Amino-3-imino-3H-xanthen-9-yl)benzoic acid methyl ester; Rh 123
$C_{21}H_{17}ClN_2O_3$ FW 380.82 [62669-70-9]

Fluorescent dye most commonly used in flow cytometry as functional reporter for P-glycoprotein (P-gp). Functional assays for MDR proteins are better prognostic indicators in cancer therapy than levels of MDR protein expression. Rh 123 can be used in multiparameter analyses without fluorescence interference in combination with common protein labeling dyes such as PE-Cy™5 and AMCA (7-amino-4-methylcoumarin-3-acetic acid). Recent reports indicate Rh 123 may also be a substrate of MRP1. Used as a laser dye and for selective cell growth effects.

EC No. 2636878 BRN 6030951

R8004	5 mg, 25 mg, 100 mg

NF-κB and Inflammation

Inflammation and Cancer: the NF-κB Connection

Chronic inflammation is an underlying factor in the development and progression of many of the chronic diseases of aging, such as arthritis, atherosclerosis, diabetes, and cancer. Oxidative cellular stress induced by environmental factors, such as cigarette smoke, UV or ionizing radiation, bacterial or viral infection, or any number of oxidizing xenobiotic compounds, triggers a wide range of cellular responses, some of which are proinflammatory and proapoptotic, while others protect the cell against apoptosis and enhance cellular adhesion, cell proliferation, and angiogenesis. The inappropriate induction or constitutive activation of these protective responses in mutated or damaged cells appears to be a major factor in the transformation and proliferation of cancer cells.[1–5]

Two nuclear transcription factors that are involved in mediating the cellular responses to oxidative cell stress and proinflammatory stimuli are activator protein-1 (AP-1) and nuclear factor-κB (NF-κB). The role of these transcription factors on cancer initiation and progression has been studied in cell culture and *in vivo* models.[5,6] The activation of two AP-1 components, c-Jun and c-Fos, by JNK and by ERK1/2 or p38 MAPK, respectively, is involved in the malignant transformation of cells stimulated by the tumor promotor phorbol 12-myristate 13-acetate (PMA). The proinflammatory and antiapoptotic response to tumor promotion is primarily mediated through activation of NF-κB by the IKK family of serine/threonine kinases. The following discussion will focus on the NF-κB pathway as a target for cancer chemotherapy and chemoprevention.

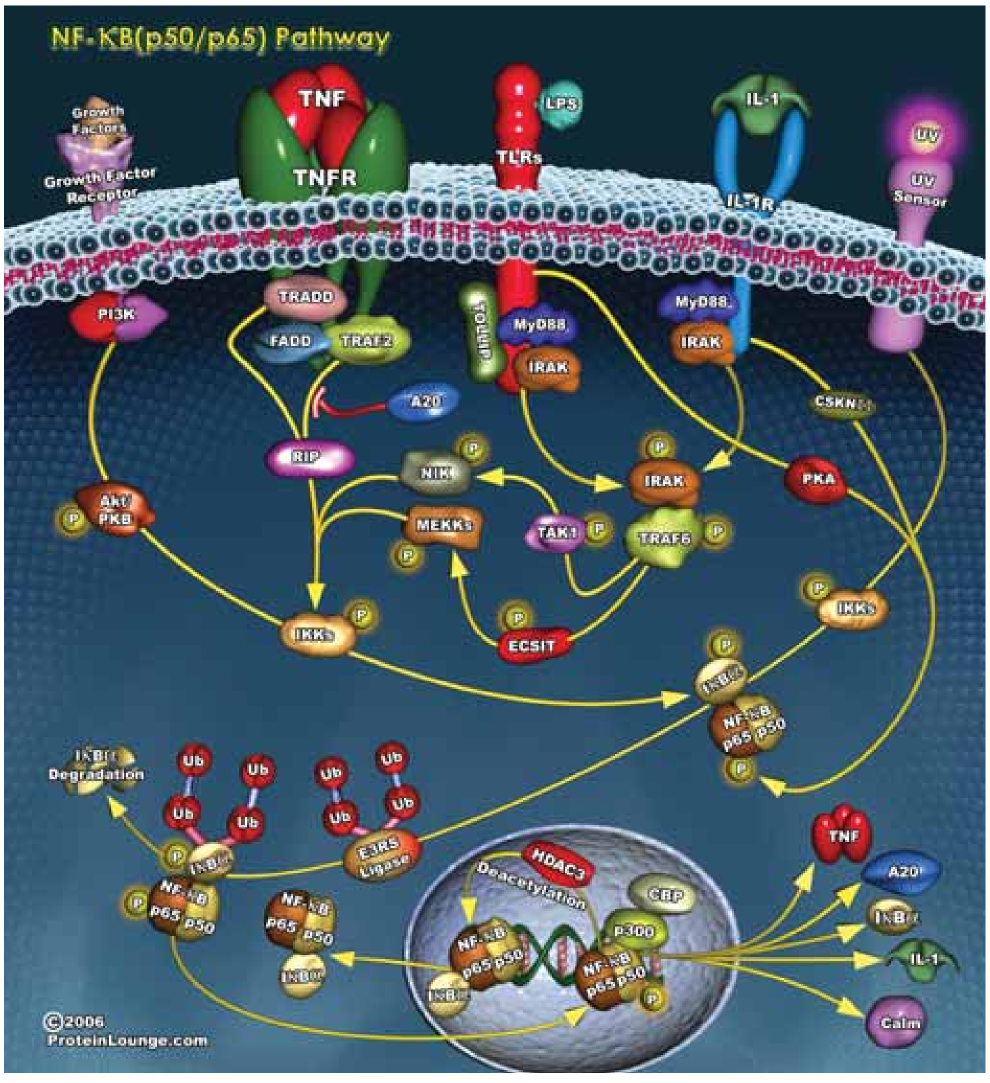

Figure 1. Many diverse stimuli utilize intracellular signaling pathways to activate NF-κB, a nuclear transcription factor that regulates proinflammatory and cell survival pathways.

NF-κB Transcription Factors

NF-κB refers to a family of transcription factors that has been highly conserved through evolution and is present in the cytoplasm of all cells. NF-κB has been called a "stress sensor" because its activity is induced by a wide variety of stimuli,[7] including tumor necrosis factor (TNF-α), PMA and other tumor promoters, cigarette smoke extract (CSE), lipopolysaccharide (LPS), oxidants, and pathogenic bacteria. The NF-κB family comprises five members: p50 (NF-κB1), p52 (NF-κB2), RelA (p65), RelB, and c-Rel. p50 and p52 are cleaved from inactive precursor proteins, p105 and p100, respectively, prior to translocation to the nucleus. NF-κB family members are characterized by having:

- a Rel homology domain that binds to DNA
- a dimerization domain
- the ability to bind to the intracellular inhibitor complex, IκB

The most widely studied NF-κB heterodimers are p50/p65 and p50/c-Rel (both associated with the classical or canonical pathway) and p52/RelB (alternative pathway). The classical pathway is activated by inflammatory cytokines, bacterial and viral infections, and oxidative stimuli and induces gene expression responsible for the antiapoptotic actions of NF-κB. The alternative pathway is primarily involved in B cell survival.[2,7–9] The classical pathway is illustrated in **Figure 1**.

Cytoplasmic NF-κB is sequestered as an inactive complex with its regulatory subunit, IκB. The most abundant member of the IκB family of proteins is IκBα. Phosphorylation of two conserved serine residues in the N-terminal domain of the NF-κB/IκB complex induces the rapid dissociation and polyubiquitination of IκB followed by its degradation by the 26S proteasome. Activated NF-κB translocates to the nucleus where specific subunit lysines are acetylated by SRC-1 and p300 histone acetyltransferases. Acetylation promotes DNA binding and NF-κB-induced gene transcription.[10] A list of more than 200 proteins that are regulated by NF-κB is given by Ahn and Aggarwal.[7] Many of the genes regulated by NF-κB code for inflammatory cytokines and proteins that mediate cell survival, cellular adhesion, cell cycle activation, cell proliferation, angiogenesis, and oncogenesis. However, not all the actions of NF-κB promote cell survival. Activation of NF-κB also appears to be essential for p53-induced apoptosis in response to oxidative stress or to the anticancer agents, doxorubicin and etoposide.[2,11]

Activation of NF-κB via Phosphorylation

Phosphorylation of the NF-κB/IκB complex is catalyzed by IKK, a protein complex that contains two homologous kinase subunits (IKKα and IKKβ) and a regulatory subunit IKKγ/NEMO.[17] Activation of the IKK complex can be initiated by any one of several intracellular phosphorylation pathways, including NF-κB-inducing kinase (TRAF/NIK),[8,12,13] MEK1,[11,14] ERK5,[15] and PI3K/Akt.[16] T cell, B cell and lysophosphatidic acid receptors activate a kinase cascade that results in the activation of the IKK complex by Bcl10, Malt1 and CARMA-1.[9,17] Acetylation of serines and threonines in the activation loop of the IKKα and IKKβ subunits can prevent phosphorylation and activation of the IKK complex.[18] In addition, regulation of the NF-κB/IκB complex can also occur independently of IKK activation or inhibition. Both the PI3K/Akt and JAK/STAT/Pim kinase pathways activate NF-κB by phosphorylating Cot, a serine-threonine kinase that can induce the proteasomal degradation of IκB.[19] Upregulation or over-expression of mitogen activated protein kinase phosphatase-5 (MKP5) can decrease cytokine-induced phosphorylation of NF-κB/IκB and of p38 MAP kinase.[20]

There are two mechanisms by which the NF-κB-induced gene transcription is terminated. Genes coding for IκB complex proteins are upregulated by NF-κB. Newly formed IκBα subunits can enter the nucleus where they bind to and inactivate NF-κB, and the NF-κB/IκBα complex is exported back to the cellular cytoplasmic compartment.[9] Alternatively, the translational action of DNA-bound NF-κB can be terminated by deacetylation of the p65 subunit at Lys^{310} by the histone deacetylases SIRT1 or HDAC1.[10,21] Inhibition of p300 histone acetyltransferase or the overexpression or activation of SIRT1 has been shown to inhibit NF-κB-mediated gene expression.[10,22] Conversely, decreased histone deacetylase level or activity has been shown to increase the expression of inflammatory cytokines, presumably through enhancement of NF-κB-mediated gene transcription.[23]

NF-κB and its Relationship to Disease

Altered regulation of NF-κB activity is observed in many genetically-linked diseases and chronic diseases of aging, including cancer.[7,9] NF-κB activation has been linked to inflammation-driven tumor promotion and progression. In addition, many solid and hematopoietic cancers express constitutively active NF-κB that contributes to the pathogenesis of the disease by inducing factors that promote proliferation, invasiveness, angiogenesis, and resistance to chemotherapeutic agents and radiation. Researchers have hypothesized that inhibition of NF-κB activation or transcriptional activity may delay cancer onset or may be used as an adjunct to more traditional chemotherapeutics. Many compounds, including many phytochemicals and micronutrients having putative chemopreventive properties, inhibit NF-κB activation or constitutive NF-κB activity in cellular or *in vivo* models of cancer.[2,5,7,24] The anticancer activities of some of these phytochemicals are summarized in the table on page 16.

Many of the inducers of NF-κB also regulate other intracellular pathways that mediate cell cycle arrest or apoptosis. One hypothesis holds that inhibition of prosurvival pathways mediated via NF-κB allows expression of proapoptotic mechanisms that may also be mediated by the various cell signaling pathways. For example, TNF-α is an inflammatory cytokine that mediates a broad spectrum of biological actions via activation of the TNFR1 receptor and, depending on the cellular environment, can promote either cell survival or programmed cell death. On the one hand, TNF-α/TNFR1-induced activation of TRAF2 initiates kinase cascades that lead to phosphorylation and activation of AP-1 and NF-κB, thereby promoting gene expression, cell survival and proliferation. Conversely, through its interactions with TRADD or FADD, TNF-α/TNFR1 initiates signaling pathways that activate caspase 8 and the proteolytic cascade that ends in apoptotic cell death. Furthermore, some of the genes induced by NF-κB activation, such as Gadd45β and XIAP, are inhibitors of prolonged JNK activation. JNK activation facilitates mitogen-induced and oxidative stress-induced apoptosis. Thus, in the presence of NF-κB inhibitors, TNFR1 activation would favor the proapoptotic, anticancer actions of TNF-α.[2,13,25]

It should be noted that many phosphorylation pathways, e.g., PI3K-Akt, JNK, and ERK, are involved in both prosurvival and proapoptotic cellular processes. Therefore, the physiological response to procarcinogenic and anticarcinogenic xenobiotics represents the sum of all the specific intracellular signaling pathways that are upregulated and down-regulated in response to these stimuli. While chemopreventive natural products may tip the balance toward cell death in some damaged or transformed cells, their actions may promote carcinogenesis or drug resistance in other cells or organisms.

NF-κB and Inflammation

Natural Product Inhibitors of NF-κB Activation

Compound	Inducer	Mechanism of NF-κB Inhibition	Chemopreventive/ Chemotherapeutic Actions	Ref.	Cat. No.	Pack Size
Betulinic acid	TNF-α, IL-1β, PMA, H_2O_2, okadaic acid, cigarette smoke	Inhibits activation of IKKα.	Downregulates COX-2 and MMP-9.	26, 27	**855057**	100 mg 500 mg
Caffeic acid phenethyl ester (CAPE)		Suppresses p65 nuclear translocalization; inhibits MAPK.	Decreases HIF-1α expression, induces HO-1 expression, reduces iNOS and MMP-9 expression.	5, 27, 28	**C8221**	1 g
Curcumin	TNF-α, PMA, H_2O_2	Inhibits phosphorylation of Akt and activation of IKKα; upregulates MKP5 expression, inhibits p38 activation.	Also inhibits STAT3 and AP-1 activation and activates PPAR-γ; suppresses expression of c-Fos and c-Jun, inhibits COX-2 expression, antioxidant; blocks tumor initiation, promotion, invasion, angiogenesis, and metastasis.	5, 20, 29, 30, 31	**C7727** ≥94% (curcuminoid content), ≥80% (Curcumin)	500 mg
					28260 Mixture of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin, ≥95.0% (TLC)	10 g 50 g
Embelin	TNF-α, LPS, IL-1β, PMA, H_2O_2, okadaic acid, cigarette smoke	Inhibits IKKα-induced phosphorylation of IκBα.	Enhances apoptosis induced by cytokines and chemotherapeutic agents.	32	**E1406**	10 mg 50 mg
Epigallocatechin gallate	PMA	Inhibits IKK activation	Also inhibits activation of AP-1, inhibits PI3K-Akt and ERK1/2 pathways, suppresses COX-2 induction, blocks *H. pylori*-induced glycosylation of TLR-4.	5, 33	**50299** ≥97.0% (HPLC)	1 mg
					E4143 ≥95%, from green tea	50 mg
Evodiamine NEW	TNF-α, LPS, IL-1β, PMA, cigarette smoke	Inhibits phosphorylation of Akt, association of Akt with IKKα, and activation of IKKα.	Inhibits COX-2 induction, enhances apoptosis induced by cytokines and chemotherapeutic agents, inhibits TNF-induced invasive activity. Inhibits both constitutive and induced NF-κB; decreases HIF-1α expression.	28, 34,35	**E3531**	250 mg
Genistein	^{60}Co Radiation, docetaxel, cisplatin		Potentiates radiation-induced apoptosis; upregulates $p21^{WAF1/Cip1}$.	36	**G6649** Synthetic, ≥98% (HPLC)	5 mg 25 mg 100 mg
					G6776 from *Glycine max* (soybean), ~98% (HPLC)	5 mg 10 mg
					91955 BioChemika, ≥98.0% (HPLC)	25 mg 100 mg
6-Gingerol NEW	TNF-α, IL-1β	Upregulates MKP5 expression, inhibits p38 activation.	Anti-inflammatory	20	**G1046**	n/a
Honokiol	TNF-α, *P. acnes*	Inhibits activation of IKKα and phosphorylation of Akt; operates downstream of MEKK-1.	Downregulates genes involved in antiapoptosis, proliferation, invasion, and angiogenesis; downregulates COX-2.	37, 38, 39	**H4914**	10 mg 25 mg
Indole-3-carbinol	TNF-α, LPS, IL-1β, PMA, H_2O_2, okadaic acid, cigarette smoke	Inhibits activation of IKKα.	Inhibits constitutive and induced NF-κB activation; binds to ERα, AhR, and AR and suppresses invasion and migration of human breast cancer cells.	40	**I7256**	1 g 5 g 25 g
Magnolol NEW	TNF-α, *P. acnes*	Inhibits activation of IKKα and phosphorylation of Akt, operates downstream of MEKK-1.	Downregulates COX-2	37,38	**M3445**	10 mg
Partenolide		Inhibits IKK		27	**P0667**	5 mg 25 mg
Piceatannol	LPS	Inhibits activation of IKKα and IKKβ, inhibits phosphorylation of Akt and Raf-1; inhibits Syk and $p56^{lck}$ activity.	Inhibits iNOS and COX-2 induction and transcription of proinflammatory cytokine genes	41,42	**P0453**	5 mg 25 mg

SIGMA

Natural Product Inhibitors of NF-κB Activation (continued)

Compound	Inducer	Mechanism of NF-κB Inhibition	Chemopreventive/ Chemotherapeutic Actions	Ref.	Cat. No.	Pack Size
Silibinin	TNF-α	Inhibits IKKα activity	Inhibits constitutive and induced NF-κB, inhibits PI3K-Akt and ERK1/2 pathways, inhibits c-Jun and c-Fos activation; inhibits tumor invasion, angiogenesis.	33, 47, 48, 49, 50	**S0417**	1 g 10 g
Tetrandrine	CD28, ConA, PHA, PMA + ionomycin	Inhibits IKKα and β activity, inhibits activation of JNK, p38 MAPK, ERK.	Antiinflammatory; also blocks AP-1 activation	51	**T2695**	1 g
Xanthohumol NEW	TNF-α		Antiangiogenic; suppresses iNOS expression.	52	**X0379**	5 mg

Abbreviations: PMA – phorbol myristate acetate; HO-1 – Heme oxygenase 1; MKP5 – mitogen activated protein kinase phosphatase-5; LPS – lipopolysaccharide; TNR-4 – Toll-like receptor-4; *P. acnes – Propionibacterium acnes*; ER-α – estogen receptor α; AhR – aryl hydrocarbon receptor; AR – androgen receptor; Con A – concanavalin A; PHA – *Phasolus vulgaris* lectin

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NF-κB and Inflammation

Antibodies and Kits

Anti-IKKα (699-715) antibody produced in rabbit

Anti-IκB Kinase α

Affinity isolated antibody

Immunogen: synthetic peptide corresponding to amino acids 699-715 of human IκB Kinase α (IKK α) (C2)[1,2]. This sequence contains one amino acid substitution with the mouse sequence.

Solution in phosphate buffered saline containing 0.02% sodium azide.

Species reactivity: human

Antigen mol wt 85 kDa

Application(s)

Immunoblotting...................... 1 µg/mL using human HeLa cell extract

Lit Cited: 1. DiDonato, J.A., et al., *Nature*, **388**, 548-554, (1997).
2. Regnier, C.H., et al., *Cell*, **90**, 373-383 (1997)

-20°C DRY ICE

I7778	0.1 mg

Anti-IKKα (716-734) antibody produced in rabbit

Anti-IκB Kinase α

Affinity isolated antibody

Immunogen: synthetic peptide corresponding to amino acids 716-734 of human IκB Kinase α (IKKα) (C1).

Solution in phosphate buffered saline containing 0.02% sodium azide.

Species reactivity: human

Antigen mol wt 85 kDa

Application(s)

Immunoblotting................... 0.5 µg/mL using human HeLa cell extract

-20°C DRY ICE

I7903	0.1 mg

Anti-IKKβ antibody produced in rabbit

Anti-IKK2; Anti-IκB Kinase β

Affinity isolated antibody

Immunogen: synthetic peptide located near the C-terminal region of human IKKβ (amino acids 720-735).

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Antigen mol wt 85 kDa

Application(s)

Immunoblotting....................................... 1:500 using a whole extract of human epitheloid carcinoma HeLa cells

-20°C DRY ICE

I9767	0.2 mL

Anti-IKKγ/NEMO (N-Terminal) antibody produced in rabbit

Anti-IKKAP1

~0.5 mg/mL, affinity isolated antibody

Immunogen: synthetic peptide corresponding to amino acids 400-416 of human IKKγ.

Solution in phosphate buffered saline containing 0.02% sodium azide.

Species reactivity: human, mouse, rat

Antigen mol wt ~52 kDa

Application(s)

Immunoblotting..0.5-1 µg/mL

-20°C DRY ICE

I5032	100 µg

Monoclonal Anti-NF-κB antibody produced in mouse

clone NF-12, ascites fluid

Immunogen: recombinant mouse NF-κB p65 fragment (C-terminal, 151 amino acids).

Contains 15 mM sodium azide

Species reactivity: mouse, human

Antigen mol wt 65 kDa

Application(s)

Immunocytochemistry.. suitable
Immunoblotting.............................. 1:1,000 using a whole cell extract from a mouse fibroblast cell line
Array ....suitable
Isotype IgG1

-20°C DRY ICE

N8523	0.2 mL

NF-κB (p50) human

Full length, 453 amino acid protein from spliced cDNA containing the p105 precursor polypeptide. For use in gel shift and footprinting assays, as well as *in vitro* transcriptional activation.

recombinant, expressed in *Escherichia coli*, buffered aqueous glycerol solution

Solution in 50 mM NaCl, 5 mM DTT, 0.5 mM PMSF, 20 mM HEPES, 10 µM zinc acetate, 0.1% NP-40, and 10% glycerol.

Unit Definition : One gel shift unit is defined as the amount of p50 required to shift 0.38 pmol of NF-κB oligonucleotide.

-70°C DRY ICE

N9909	50 un

NEW Histone Deacetylase Assay Kit, Fluorometric

Histone deacetylases (HDACs) are enzymes that remove acetyl groups from histone proteins. HDACs are important regulators of gene expression and genome function. They are implicated in a number of human disease states, namely several cancers, neurological disorders, and aging. Therefore, HDAC are relevant key targets for therapeutic intervention. Moreover, histone deacetylase inhibitors have been shown to serve as antitumor agents.

The Histone Deacetylase Assay Kit provides a simple method for the detection of HDAC activity based on a two-step enzymatic reaction. The substrate for this enzymatic assay is a substituted peptide with an acetylated lysine residue and a bound fluorescent group. The first step of the reaction is deacetylation of the acetylated lysine side chain by the HDAC containing sample (HeLa cell extract, purified enzyme, etc.). The second step is the cleavage of the deacetylated substrate by the developer solution and the release of the free highly fluorescent group. The measured fluorescence is directly proportional to the deacetylation activity of the sample.

The Histone Deacetylase Assay Kit has been tested on HeLa, HEK 293T, NIH 3T3, and U 973 cell extracts. The kit includes all the reagents required for the fast and easy measurement of HDAC activity in cell or nuclear extracts, or with purified enzyme preparations. In addition, the kit provides HeLa cell lysate as a source of HDAC activity for inhibitor screening or as a positive control, a HDAC inhibitor (Trichostatin A), and a standard to enable activity quantitation.

Sufficient for 100 assays (96 well plates)

-20°C DRY ICE

CS1010	1 kit

NEW Sirt1 human

SIR2α; SIR2L1; Sirtuin1

Sirtuins are a family of NAD^+ dependent deacetylases that remove an acetyl group from the ε-amino group of lysine residues. The proteins within this family are named after the first protein discovered, from yeast, called Sir2 (**S**ilent **I**nformation **R**egulator 2). The proteins are conserved from bacteria to higher eukaryotes. In humans, there are seven Sir2 family members (SIRT1 to SIRT7). SIRT1 plays a pivotal role in the regulation of cellular differentiation, metabolism, cell cycle, apoptosis and regulation of p53. Several targets for SIRT1 were identified, among them Lys^{382} of p53.[1] Using RNA interference, additional targets were identified. It was demonstrated that reduced levels of human SIRT1 led to increased acetylation of Histone $H4\text{-}Lys^{16}$, $H4\text{-}Lys^{20}$, and Histone $H3\text{-}Lys^{9}$ as well as histone $H1\text{-}Lys^{26}$.[2]

Recombinant, expressed in *Escherichia coli*, N-terminal histidine tagged, >90% (SDS-PAGE)

Solution containing 50 mM Tris, pH 7.4, 100 mM NaCl, 1 mM DTT, protease inhibitors (Cat. No. P8340) 1:200, and 10% glycerol (w/v).

Lit cited: 1. Vaziri, H., et al., *Cell* **107**, 149-159 (2001)
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-20°C DRY ICE

S8446	150 µg

Monoclonal Anti-Sirt1 antibody produced in mouse

Anti-mouse homologue of yeast Sir2; Anti-Sir2α; Anti-Sirtuin1

~2 mg/mL, clone SIR11, purified immunoglobulin

Immunogen: synthetic peptide corresponding to amino acids 722-737 at the C-terminus of mouse Sir2 with C-terminal added lysine.

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Species reactivity: mouse

Antigen mol wt ~105 kDa

Application(s)

Immunoblotting............. 1-2 µg/mL using total cell extract of C-2 cells
Indirect ELISA.............suitable
Array.............suitable
Isotype.............IgG1

-20°C DRY ICE

S5196	200 µL

NEW SIRT1 Assay Kit

The assay procedure is based on a two-step enzymatic reaction. The first step is deacetylation by SIRT1 of a substrate that contains an acetylated lysine side chain. The second step is the cleavage of the deacetylated substrate by the Developing Solution and the release of a highly fluorescent group. The measured fluorescence is directly proportional to the deacetylation activity of the enzyme in the sample. The kit offers all the reagents required for the fast and easy measurement of purified SIRT1 activity and for screening of inhibitors/activators. Moreover, the kit contains an inhibitor (nicotinamide) and an activator (resveratrol) as negative and positive controls, respectively.

Sufficient for 100 assays

Components

Assay Buffer ....20 mL
SIRT1 Substrate (Fluorometric) ....100 µL
Standard (non-acetylated) 20 mM ....100 µL
Developer Solution ....1.5 mL
Nicotinamide Solution (inhibitor) ....100 µL
Sirt1 human ....150 µg
NAD^+ Solution ....1 mL
Resveratrol Solution (activator) ....100 µL

-20°C DRY ICE

CS1040	1 kit

Anti-Sir2 (AS-16) antibody produced in rabbit

Affinity isolated antibody

Immunogen: synthetic peptide corresponding to mouse Sir2 sequence (amino acids 722-737) with N-terminal cysteine added conjugated to KLH. This sequence is 62% homologous to the corresponding human sequence.

Solution in 0.01 M phosphate buffered saline, pH 7.4 containing 1% bovine serum albumin and 15 mM sodium azide

Antigen mol wt ~110 kDa

Species reactivity: mouse

Application(s)

Array.............suitable
Immunoblotting.............suitable using mouse NIH 3T3 cells
Immunoprecipitation.............suitable
Indirect immunofluorescence.............suitable using mouse NIH 3T3 cells

-20°C DRY ICE

S5313	0.2 mL

Splitomicin

≥98% (HPLC)

Sir2p (silent information regulator) and HDAC inhibitor.

2–8°C

S4068	5 mg

DNA Damage and Repair

DNA Damage and Repair Mechanisms

Damage to cellular DNA is involved in mutagenesis and the development of cancer. The DNA in a human cell undergoes several thousand to a million damaging events per day, generated by both external (exogenous) and internal metabolic (endogenous) processes. Changes to the cellular genome can generate errors in the transcription of DNA and ensuing translation into proteins necessary for signaling and cellular function. Genomic mutations can also be carried over into daughter generations of cells if the mutation is not repaired prior to mitosis.

Once cells lose their ability to effectively repair damaged DNA, there are three possible responses (see **Figure 1**).

1. The cell may become senescent, i.e., irreversibly dormant. In 2005, multiple laboratories reported that senescence could occur in cancer cells *in vivo* as well as *in vitro*, stopping mitosis and preventing the cell from evolving further.[1-4]
2. The cell may become apoptotic. Sufficient DNA damage may trigger an apoptotic signaling cascade, forcing the cell into programmed cell death.
3. The cell may become malignant, i.e., develop immortal characteristics and begin uncontrolled division.

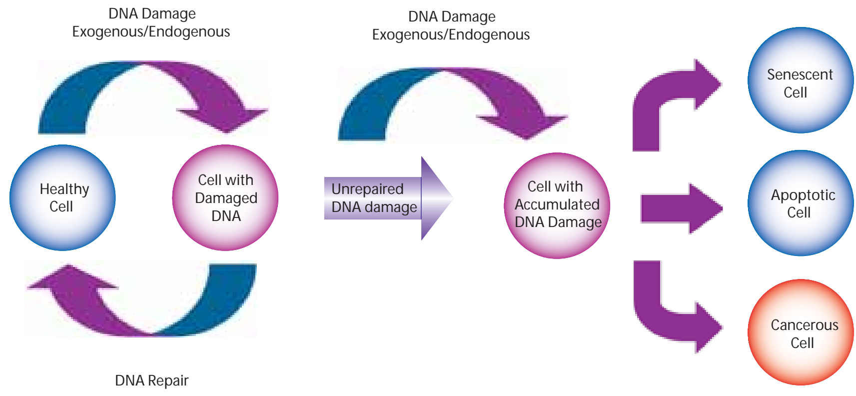

Figure 1. The pathway of cellular DNA damage and repair that leads to senescence, apoptosis, or cancer.

To compensate for the degree and types of DNA damage that occur, cells have developed multiple repair processes including mismatch, base excision, and nucleotide excision repair mechanisms, with little process redundancy. Cells may have evolved to proceed into apoptosis or senescence if overwhelming damage occurs rather than expend energy to effectively repair the damage. The rate at which a cell is able to make repairs is contingent on factors including cell type and cell age.

Sources of DNA Damage

For many years, exogenous sources of damage have been thought to be the primary cause of DNA mutations leading to cancer. However, Jackson and Loeb proposed that endogenous sources of DNA damage also contribute significantly to mutations that lead to malignancy.[5] Both environmental and cellular sources can result in similar types of DNA damage.

DNA can be attacked by physical and chemical mutagens. Physical mutagens are primarily radiation sources, including UV (200-300 nm wavelength) radiation from the sun. UV radiation produces covalent bonds that crosslink adjacent pyrimidine (cytosine and thymine) bases in the DNA strand. Ionizing radiation (X-rays) initiates DNA mutations by generating free radicals within the cell that create reactive oxygen species (ROS) and result in single-strand and double-strand breaks in the double helix. Chemical mutagens can attach alkyl groups covalently to DNA bases; nitrogen mustard compounds that can methylate or ethylate the DNA base are examples of DNA alkylating agents. Procarcinogens are chemically inert precursors that are metabolically converted into highly reactive carcinogens. These carcinogens can react with DNA by forming DNA adducts, i.e., chemical entities attached to DNA. Benzo*[a]*pyrene, a polyaromatic heterocycle, is not itself carcinogenic. It undergoes two sequential oxidation reactions mediated by cytochrome P450 enzymes, which results in benzo*[a]*pyrenediol epoxide (BPDE), the carcinogenic metabolite that is able to form a covalent DNA adduct (see **Figure 2**).

CYP + NADPH + O_2 → + H_2O → CYP + NADPH + O_2 →

Benzo*[a]*pyrene (procarcinogen) — Benzo*[a]*pyrenediolepoxide (carcinogen)

Figure 2. Benzo*[a]*pyrene is oxidized by P450 enzymes to create the highly carcinogenic benzo*[a]*pyrenediolepoxide.

DNA damage can also result from endogenous metabolic and biochemical reactions, some of which are not well understood.[6] Hydrolysis reactions can partially or completely cleave the nucleotide base from the DNA strand. The chemical bond connecting a purine base (adenine or guanine) to the deoxyribosyl phosphate chain can spontaneously break in the process known as depurination. An estimated 10,000 depurination events occur per day in a mammalian cell.[7] Depyrimidination (loss of pyrimidine base from thymine or cytosine) also occurs, but at a rate 20 to 100-fold lower than depurination.

Deamination occurs within the cell with the loss of amine groups from adenine, guanine, and cytosine rings, resulting in hypoxanthine, xanthine, and uracil, respectively. DNA repair enzymes are able to recognize and correct these unnatural bases. However, an uncorrected uracil base may be misread as a thymine during subsequent DNA replication and generate a C→T point mutation.

DNA methylation, a specific form of alkylation, occurs within the cell due to a reaction with S-adenosylmethionine (SAM). SAM is an intracellular metabolic intermediate that contains a highly reactive methyl group. In mammalian cells, methylation occurs at the 5-position of the cytosine ring of a cytidine base (C) that is 5′ to a guanosine base (G), i.e., sequence CpG. A significant source of mutation error is the spontaneous deamination of the 5-methylcytosine product of methyl-ation. Loss of the amine group results in a thymine base, which is not detected by DNA repair enzymes as an unnatural base. The resulting substitution is retained in DNA replication, creating a C→T point mutation (see **Figure 3**).

Figure 3. The 2-phase mutation of cytosine results in thymine, creating a C→T point mutation.

Normal metabolic processes generate reactive oxygen species (ROS), which modify bases by oxidation. Both purine and pyrimidine bases are subject to oxidation. The most common mutation is guanine oxidized to 8-oxo-7,8-dihydroguanine, resulting in the nucleotide 8-oxo-deoxyguanosine (8-oxo-dG). The 8-oxo-dG is capable of base pairing with deoxyadenosine, instead of pairing with deoxycytotidine as expected. If this error is not detected and corrected by mismatch repair enzymes, the DNA subsequently replicated will contain a C→A point mutation. ROS may also cause depurination, depyrimidination, and single-strand or double strand breaks in the DNA.

Other genomic mutations may be introduced during DNA replication in the S phase of the cell cycle. Polymerases that duplicate template DNA have a small but significant error rate, and may incorporate an incorrect nucleotide based on Watson-Crick pairing versus the template DNA. Chemically altered nucleotide precursors may be incorporated into the generated DNA by the polymerase, instead of normal bases. In addition, polymerases are prone to "stuttering" when copying sections of DNA that contain a large number of repeating nucleotides or repeating sequences (microsatellite regions). This enzymatic "stuttering" is due to a strand slippage, when the template and replicated strands of DNA slip out of proper alignment. As a result, the polymerase fails to insert the correct number of nucleotides indicated by the template DNA, resulting in too few or too many nucleotides in the daughter strand.

Single strand and double strand cleavage of the DNA may occur. Single strand breaks may result from damage to the deoxyribose moiety of the DNA deoxyribosylphosphate chain. Breaks also result as an intermediate step of the base excision repair pathway after the removal of deoxyribose phosphate by AP-endonuclease 1.[8] When a single strand break occurs, both the nucleotide base and the deoxyribose backbone are lost from the DNA structure. Double strand cleavage most often occurs when the cell is passing through S-phase, as the DNA may be more susceptible to breakage while it is unraveling for use as a template for replication.

Mechanisms of DNA Repair

While the cell is able to evolve into either an apoptotic or senescent state, these actions are performed as a last resort. For each type of DNA damage, the cell has evolved a specific method of repairing the damage or eliminating the damaging compound.

O^6-Methylguanine DNA methyltransferase (MGMT; DNA alkyltransferase) cleaves both methyl and ethyl adducts from guanine bases on the DNA structure. The reaction is not a catalytic (enzymatic) reaction but is stoichiometric (chemical), consuming one molecule of MGMT for each adduct removed. Cells that have been engineered to overexpress MGMT are more resistant to cancer, likely because they are able to negate a larger amount of alkylating damage. A recent study by Niture, et al., reports an increase in MGMT expression by use of cysteine/glutathione enhancing drugs and natural antioxidants.[9]

DNA polymerases such as polymerase-δ contain proofreading activities and are primarily involved in replication error repair. When an error is detected, these polymerases halt the process of DNA replication, work backward to remove nucleotides from the daughter DNA chain until it is apparent that the improper nucleotide is gone, and then reinitiate the forward replication process. Mice with a point mutation in both copies of the *Pold*1 gene demonstrated a loss of proofreading activity by DNA polymerase-δ and developed epithelial cancers at a significantly higher rate than did mice with wild-type genes or with a single copy mutation.[10]

A group of proteins known as mismatch excision repair (MMR) enzymes is capable of correcting errors of replication not detected by the proofreading activities of DNA polymerase. MMR enzymes excise an incorrect nucleotide from the daughter DNA and repair the strand using W-C pairing and the parent DNA strand as the correct template.[11] This is especially crucial for errors generated during the replication of microsatellite regions, as the proofreading activity of DNA polymerase does not detect these errors. To a lesser degree, MMR enzymes also correct a variety of base pair anomalies resulting from DNA oxidation or alkylation. These mutations include modified base pairs containing O^6-methylguanine and 8-oxoguanine, and carcinogen and cisplatin adducts.[12,13] Mutations in the human mismatch excision repair genes *MSH2* and *MLH1* are associated with hereditary non-polyposis colorectal cancer (HNPCC) syndrome.[14]

DNA Damage and Repair

Base Excision Repair and Nucleotide Excision Repair

Base excision repair (BER) involves multiple enzymes to excise and replace a single damaged nucleotide base. The base modifications primarily repaired by BER enzymes are those damaged by endogenous oxidation and hydrolysis. A DNA glycosylase cleaves the bond between the nucleotide base and ribose, leaving the ribose phosphate chain of the DNA intact but resulting in an apurinic or apyrimidinic (AP) site. 8-Oxoguanine DNA glycosylase I (Ogg1) removes 7,8-dihydro-8-oxoguanine (8-oxoG), one of the base mutations generated by reactive oxygen species. Polymorphism in the human *OGG1* gene is associated with the risk of various cancers such as lung and prostate cancer. Uracil DNA glycosylase, another BER enzyme, excises the uracil that is the product of cytosine deamination, thereby preventing the subsequent C→T point mutation.[15] N-Methylpurine DNA glycosylase (MPG) is able to remove a variety of modified purine bases.[16]

The AP sites in the DNA that result from the action of BER enzymes, as well as those that result from depyrimidination and depurination actions, are repaired by the action of AP-endonuclease 1 (APE1). APE1 cleaves the phosphodiester chain 5′ to the AP site. The DNA strand then contains a 3′-hydroxyl group and a 5′-abasic deoxyribose phosphate. DNA polymerase β (Polβ) inserts the correct nucleotide based on the corresponding W-C pairing and removes the deoxyribose phosphate through its associated AP-lyase activity. The presence of X-ray repair cross-complementing group 1 (XRCC1) is necessary to form a heterodimer with DNA ligase III (LIG3). XRCC1 acts as a scaffold protein to present a non-reactive binding site for Polβ, and bring the Polβ and LIG3 enzymes together at the site of repair.[17] Poly(ADP-ribose) polymerase (PARP-1) interacts with XRCC1 and Polβ and is a necessary component of the BER pathway.[18,19] The final step in the repair is performed by LIG3, which connects the deoxyribose of the replacement nucleotide to the deoxyribosylphosphate backbone. This pathway has been named "short-patch BER".[20]

An alternative pathway called "long-patch BER" replaces a strand of nucleotides with a minimum length of 2 nucleotides. Repair lengths of 10 to 12 nucleotides have been reported.[21,22] Long-patch BER requires the presence of proliferation cell nuclear antigen (PCNA), which acts as a scaffold protein for the restructuring enzymes.[23] Other DNA polymerases, possibly Polδ and Polε, [24] are used to generate an oligonucleotide flap. The existing nucleotide sequence is removed by flap endonuclease-1 (FEN1). The oligonucleotide is then ligated to the DNA by DNA ligase I (LIG1), sealing the break and completing the repair.[17] The process used to determine the selection of short-patch versus long patch BER pathways is still under investigation (see **Figure 4**).[25]

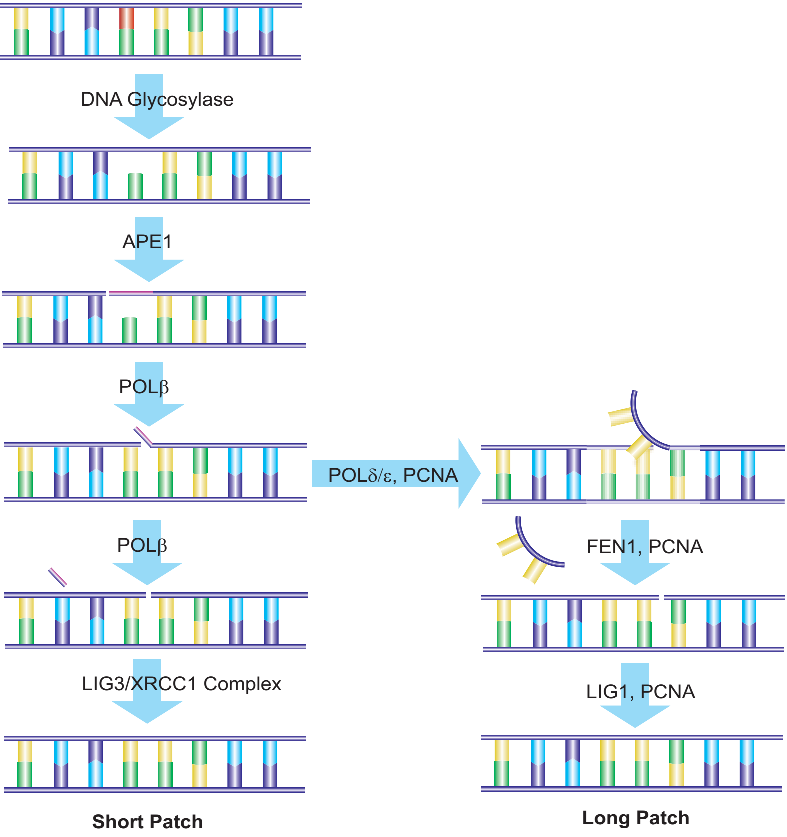

Figure 4. Schematic of both short-patch and long-patch BER pathways.

While BER may replace multiple nucleotides via the long-patch pathway, the initiating event for both short-patch and long-patch BER is damage to a single nucleotide, resulting in minimal impact on the structure of the DNA double helix. Nucleotide excision repair (NER) repairs damage to a nucleotide strand containing at least 2 bases and creating a structural distortion of the DNA. NER acts to repair single strand breaks in addition to serial damage from exogenous sources such as bulky DNA adducts and UV radiation.[26] The same pathway may be used to repair damage from oxidative stress.[27] Over 20 proteins are involved in the NER pathway in mammalian cells, including XPA, XPC-hHR23B, replication protein A (RPA), transcription factor TFIIH, XPB and XPD DNA helicases, ERCC1-XPF and XPG, Polδ, Polε, PCNA, and replication factor C.[28] Overexpression of the excision repair cross-complementing (*ERCC*1) gene has been associated with cisplatin resistance by non-small-cell lung cancer cells[29] and corresponds to enhanced DNA repair capacity.[30] Global genomic NER (GGR) repairs damage throughout the genome, while a specific NER pathway called Transcription Coupled Repair (TCR) repairs genes during active RNA polymerase transcription.[31]

Repair of Double-Strand Breaks

Double-strand breaks in DNA can result in loss and rearrangement of genomic sequences. These breaks are repaired by either non-homologous end-joining (NHEJ) or by homologous recombination (HR), also called recombinational repair or template–assisted repair.

The HR pathway is activated when the cell is in late S/G_2 phase and the template has recently been duplicated. This mechanism requires the presence of an identical or nearly identical sequence linked to the damaged DNA region via the centromere for use as a repair template. Double-stranded breaks repaired by this mechanism are usually caused by the replication machinery attempting to synthesize across a single-strand break or unrepaired lesion, resulting in the collapse of the replication fork.

Non-homologous end-joining (NHEJ) is used at other points of the cell cycle when sister chromatids are not available for use as HR templates. When these breaks occur, the cell has not yet replicated the region of DNA that contains the break, so unlike the HR pathway, there is no corresponding template strand available. In NHEJ, the Ku heterodimeric protein positions the two ends of the broken DNA strands for repair without an available template, losing sequence information in the process. Multiple enzymes are involved in the rejoining process, including DNA ligase IV, XRCC4, and DNA-dependent protein kinase (DNA-PK).[32,33] NHEJ is inherently mutagenic as it relies on chance pairings, called microhomologies, between the single-stranded tails of the two DNA fragments to be joined (see **Figure 5**). In higher eukaryotes, DNA-PK is required for NHEJ repair, both via the primary mechanism and via an alternative back-up mechanism (D-NHEJ).[34]

Future Applications

While DNA damage is a key factor in the development and evolution of cancer cells, continued damage is used as part of clinical treatments for cancer, forcing malignant cells into apoptosis or senescence. Many chemotheraputic drugs such as bleomycin, mitomycin, and cisplatin, are effective because they cause further DNA damage in cancer cells that replicate at a faster rate than surrounding tissue. Cellular DNA repair mechanisms are a double-edged sword; by reducing mutations that may lead to cancer, these processes strive for genomic integrity, but the same mechanisms in malignant cells allow those cells to survive additional DNA damage and continue uncontrolled growth. In order to block this survival mechanism within cancer cells, clinical trials are now being performed using inhibitors to specific DNA repair enzymes, including MGMT, PARP, and DNA-PK.[35-38]

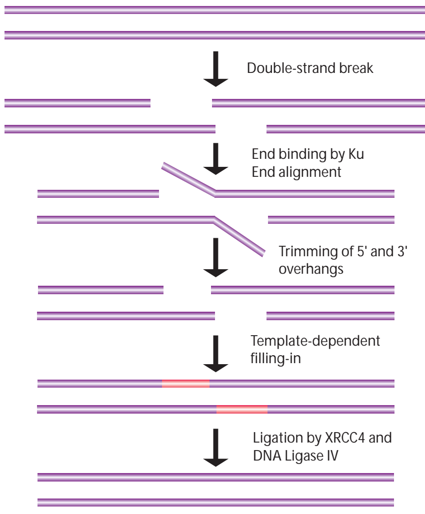

Figure 5. General mechanism of NHEJ repair of double-strand breaks in DNA.

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DNA Damage and Repair

DNA Alkylating Agents

Compound	Synonyms	Biological Action	Cat. No.	Description	Pack Size
Angelicin		Upon long wavelength UV irradiation, forms monoadduct with double-stranded DNA; inhibits DNA and RNA synthesis and cell replication in Ehrlich ascites tumor cells.	**A0956**		10 mg 25 mg
Azoxymethane	AOM	Carcinogen that induces O^6-methylguanine adducts in DNA leading to G to A transitions. Induces colon tumors in laboratory animals; used to study the mechanism of cancer progression and chemoprevention.	**A2853**	13.4 M, ≥90% (GC), contains ≤15% ethanol and acetic acid	25 mg 100 mg
Benzo*[a]*pyrene	Benzo*[def]*chrysene; 3,4-Benzopyrene; 3,4-Benzpyrene	A polycyclic aromatic hydrocarbon (PAH), environmental pollutant and carcinogen. Forms depurinating adducts with DNA that dissociate to form abasic lesions.	**B1760**	≥96% (HPLC)	1 g
Bleomycin sulfate from *Streptomyces verticillus*	Blenoxane; Bleo; Blexane	Anticancer antibiotic that binds to DNA, inhibits DNA synthesis and causes DNA scissions at specific base sequences. Needs to bind oxygen and a metal ion such as copper or iron to cleave DNA. Metallobleomycin conjugates also cleave tRNA and DNA-RNA hybrids.	**B5507**	Activity: 1.2-1.7 units/mg solid A mixture of bleomycin sulfate salts Cu <0.1%	15 units
			B2434	Biotechnology Performance Certified Cell culture tested Potency: 1.5-2.0 units/mg	20 mg
Chlorambucil	4-[Bis(2-chloroethyl)amino] benzenebutyric acid	Anticancer agent that alkylates DNA and induces apoptosis by a p53-dependent mechanism.	See **C0253** on page 9		
Daunorubicin hydrochloride	Daunomycin hydrochloride	Anthracycline anticancer antibiotic. Induces damage of DNA by intercalating into DNA and inhibiting topoisomerase II.	See **D8809** on page 9		
3,3′-Dichlorobenzidine dihydrochloride	DCB	Industrial carcinogen that induces liver and bladder tumors in laboratory animals.	**D9886**		25 g
7,12-Dimethyl-benz*[a]*anthracene	9,10-Dimethyl-1,2-benzanthracene; DMBA	Carcinogen that is activated to form covalent adducts with DNA. Used to induce colon, skin and mammary tumors in mice.	**D3254**	≥95%	100 mg 1 g 5 g
N,N′-Dimethylhydrazine dihydrochloride	1,2-Dimethylhydrazine dihydrochloride; DMH; N,N-Dimethylhydrazine (sym.) dihydrochloride	Methylating agent that induces O^6-methyl-guanine adducts in DNA; induces colon cancer in rats and mice.	**D161802**	≥99%	1 g 10 g
Doxorubicin hydrochloride	Adriamycin hydrochloride; DOX; Hydroxydaunorubicin hydrochloride	Anthracycline anticancer antibiotic. Induces damage of DNA by intercalating into DNA and inhibiting topoisomerase II.	See **D1515** on page 9		
L-Ethionine	L-2-Amino-4-(ethylthio)-butyric acid	An analog of methionine that interferes with the normal methylation of DNA and other methylation pathways, and induces pancreatic toxicity and liver cancer. However, in animal studies, it has also been shown to act synergistically with methionine-depletion to block the growth and metastasis of methionine-dependent tumors.	**E1260**	~99% (TLC)	250 mg 1 g
N-(2-Fluorenyl)acetamide	2-AAF; 2-Acetamido-fluorene; N-Acetyl-2-aminofluorene	A genotoxic carcinogen that forms adducts with DNA following its N-hydroxylation by CYP1A2; used to model liver carcinogenesis in rat.	**A7015**	≥90% (TLC)	5 g 25 g
Hexestrol	*meso*-3,4-Bis(4-hydroxy-phenyl)hexane; 4,4′-(1,2-Diethylethylene)diphenol; Dihydrodiethylstilbestrol	Nonsteroidal synthetic estrogen; reduced analog of diethylstilbestrol. Hexestrol is metabolized to its quinone, which forms depurinating adducts with DNA leading to oncogenic mutations and cancer initiation.	**H7753**	≥98%	1 g 5 g
Idarubicin hydrochloride	DMDR; Idamycin; IMI-30	Anthracycline anticancer antibiotic. Groove-binding intercalator of DNA with higher capacity than daunorubicin; topoisomerase II inhibitor.	**I1656**		10 mg
Methoxyamine hydrochloride	Methoxylamine hydrochloride; O-Methylhydroxylamine hydrochloride	Binds to abasic sites on DNA and acts as an inhibitor of base excision repair.	**226904**	98%	1 g 5 g 25 g
Methyl methanesulfonate	Methanesulfonic acid methyl ester	Randomly methylates DNA in both the major (7-MeG) and minor (3-MeA and 3-MeG) grooves.	**M4016**	~99% (GC)	1 g 5 g 25 g

DNA Alkylating Agents (continued)

Compound	Synonyms	Biological Action	Cat. No.	Description	Pack Size
Mitoxantrone dihydrochloride	1,4-Dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)-amino]ethyl]-amino]-9,10-anthracenedione dihydrochloride	DNA intercalating drug. Inhibits DNA synthesis.			See **M6545** on page 9
N-Nitrosodiethylamine	Diethylnitrosamine; NDEA	Ethylating agent that is carcinogenic in all animal species tested. Main target organs are the nasal cavity, trachea, lung, esophagus and liver.	**N0258**	ISOPAC®[1]	1 g
			N0756	Bulk Package	10 mL 25 mL
N-Nitroso-N-ethylurea	ENU; N-Ethyl-N-nitroso-urea	DNA alkylating agent that is carcinogenic in many animal species. Induces benign and malignant tumors in numerous organs, including nervous system, stomach, esophagus, pancreas, respiratory tract, intestine, lymphoreticular tissues, skin and kidney.	**N3385**	ISOPAC®[1]	1 g
			N8509	Bulk Package Contains aqueous acetic acid as stabilizer Weight on dry basis	5 g 25 g
N-Nitroso-N-methylurea	MNU; N-Methyl-N-nitroso-urea	DNA alkylating agent that is carcinogenic in many animal species. Induces benign and malignant tumors in numerous organs, including nervous system, stomach, esophagus, pancreas, respiratory tract, intestine, lymphoreticular tissues, skin and kidney.	**N1517**	ISOPAC®[1]	1 g
			N4766	Bulk Package Contains aqueous acetic acid as stabilizer Weight on dry basis	25 g 100 g
1-Nitrosopiperidine	NPIP	Carcinogen that induces benign and malignant tumors of the respiratory tract, stomach and esophagus in rodents. Potent nasal carcinogen in rats.	**N6007**		5 mL
1-Nitrosopyrrolidine	N-Nitrosopyrrolidine; NPYR	Carcinogen that forms DNA adducts that primarily result in A:T to G:C mutations. Induces hepatocellular carcinomas and lung adenomas in mice.	**158240**	99%	10 g
Ochratoxin A	N-[(3R)-(5-Chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl)carbonyl]-L-phenylalanine	Mycotoxin found in food that is nephrotoxic and carcinogenic in the kidney. Its metabolites are genotoxic and form adducts with DNA in kidney cells from several species.	**O1877**	from *Aspergillus ochraceus (Aspergillus oryzae)*, ≥98% (TLC) Benzene-Free	1 mg 5 mg 25 mg
2,3,4,5,6-Pentafluoro-benzyl bromide	α-Bromo-2,3,4,5,6-penta-fluorotoluene	Used to derivatize *N*-7-substituted guanine adducts of DNA for determination by GC-electron capture mass spectrometry	**101052**	99%	1 g 5 g 25 g
Rebeccamycin		Rebeccamycin intercalates into the DNA and inhibits topoisomerase I by stabilizing the covalent topoisomerase I-DNA intermediate (the cleavable complex) resulting in increased DNA strand breaks.	**R4900**	≥98% (HPLC), from *Saccharothrix aerocolonigenes*	250 µg
Safrole	5-Allyl-1,3-benzodioxole; 4-Allyl-1,2-methylene-di-oxybenzene	Genotoxic hepatocarcinogen; metabolites of safrole form adducts with DNA and induce chromasomal aberrations and sister chromatid exchanges.	**S9652**	≥97%	50 mL 500 mL

1. ISOPAC® products are packaged in 100 mL serum bottles with a butyl rubber stopper and aluminum tear seal. Injecting any compatible solvent permits preparation of any desired strength solution without exposure.

SIGMA

DNA Damage and Repair

DNA Crosslinking Agents

Compound	Synonyms	DNA Crosslinking Action	Cat. No.	Description	Pack Size
4′-Aminomethyltrioxsalen hydrochloride	4′-Aminomethyl-4,5′,8-trimethylpsoralen hydrochloride	Used to inactivate DNA and RNA viruses, including HIV-1, by nucleic acid crosslinking followed by UV irradiation.	**A4330**	≥98%	5 mg
Carboplatin	*cis*-Diammine(1,1-cyclo-butanedicarboxylato) platinum	A platinum-based antineoplastic drug that forms intrastrand crosslinks with neighboring guanine residues in DNA. Tumors acquire resistance to these drugs through the loss of DNA-mismatch repair (MMR) activity.	**C2538**	≥98% (HPLC)	100 mg 250 mg
Carmustine	BCNU; 1,3-Bis(2-chloro-ethyl)-1-nitrosourea	DNA alkylating agent causing DNA interstrand crosslinks.	See **C0400** on page 9		
CB 1954	5-(1-Aziridinyl)-2,4-di-nitrobenzamide	Anticancer prodrug that is activated by nitroreductase to form a potent DNA crosslinking agent.	**C2235**		50 mg
Cyclophosphamide	Cytoxan	Cytotoxic nitrogen mustard derivative used in cancer chemotherapy. Cyclophosphamide crosslinks DNA, causes strand breakage, and induces mutations.	See **C0768, C7397** on page 9		
cis-Diammineplatinum(II) dichloride	Cisplatin	Potent platinum-based antineoplastic agent. Forms cytotoxic adducts with the DNA dinucleotide d(pGpG), inducing intrastrand crosslinks.	See **P4394** on page 9		
Melphalan	4-[Bis(2-chloro-ethyl)amino]-L-phenyl-alanine; L-PAM; L-Phenyl-alanine mustard	Antineoplastic agent that forms DNA intrastrand crosslinks by bifunctional alkylation of 5′-GGC sequences.	**M2011**	≥95% (TLC)	100 mg 250 mg 1 g
Mitomycin C	MMC	Following enzymatic reduction, activated mitomycin C forms covalent adducts and crosslinks with CpG sequences in DNA.	See **M0503** on page 9		
Nimustine hydrochloride	N′-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]-N-(2-chloroethyl)-N-nitrosourea hydrochloride; ACNU	Antineoplastic compound that is a bifunctional DNA alkylating agent.	**N8659**		1 g
Oxaliplatin	[SP-4-2-(1R-*trans*)]-(1,2-Cyclohexanediamine-N,N′)[ethanedioata (2–)-O,O′] platinum	Platinum-based antitumor agent that forms interstrand crosslinks with neighboring guanine residues in DNA.	**O9512**		5 mg
Psoralen	7H-Furo[3,2-*g*] benzopyran-7-one; Furo[3,2-*g*]coumarin	When activated by UV light, psoralen induces interstrand crosslinks in DNA. Photochemical reagent for the investigation of nucleic acid structure and function.	**P8399**	≥99%	10 mg 25 mg 100 mg
Trioxsalen	TMP; 4,5′,8-Trimethyl-psoralen; Trisoralen	Photochemical crosslinker of DNA has been used as a probe for nucleic acid structure and function.	**T6137**	≥98% (HPLC)	100 mg 500 mg 1 g

SIGMA

DNA Synthesis Inhibitors

Compound	Synonyms	Inhibition Specificity	Cat. No.	Description	Pack Size
Aminopterin	4-Aminofolic acid; 4-Amino-PGA; 4-Amino-pteroyl-L-glutamic acid	Blocks DNA synthesis by blocking the production of tetrahydrofolate cofactors required for the synthesis of thymidine. In the cell, it is converted by folylpolyglutamate synthase to a high molecular weight polyglutamate metabolite that binds to dihydrofolate reductase and inhibits its activity. Aminopterin is more potent but more toxic than methotrexate.	**A1784**	~98% (TLC)	50 mg 100 mg 1 g
Methotrexate hydrate	(+)-Amethopterin hydrate; 4-Amino-10-methylfolic acid; Methylaminopterin; MTX		See **A6770** on page 9		
Cytosine β-D-arabinofuranoside	Arabinocytidine; (β-D-Arabinofuranosyl)cytosine; Arabinosylcytosine; Ara-C; Cytarabine; Cytosine arabinoside	Ara-C incorporates into DNA and inhibits DNA replication by forming cleavage complexes with topoisomerase I resulting in DNA fragmentation; does not inhibit RNA synthesis.	**C1768**	Free base ≥90% (HPLC)	100 mg 500 mg 1 g 5 g
			C6645	Hydrochloride ≥99% (HPLC)	25 mg 100 mg 500 mg 1 g 5 g
5-Fluoro-5′-deoxyuridine	5′dFUrd; Doxifluridine	5-Fluoro-5′-deoxyuridine is a prodrug that is converted to 5-fluorouracil by uridine phosphorylase.	**F8791**	≥98% (TLC)	25 mg 100 mg 250 mg
5-Fluorouracil	2,4-Dihydroxy-5-fluoro-pyrimidine; 5-Fluoro-2,4(1*H*,3*H*)-pyrimidine-dione; 5-FU	5-Fuorouracil (5-FU) is a potent antitumor agent that inhibits thymidylate synthetase thus depleting intracellular dTTP pools. 5-FU is metabolized to ribonucleotides and deoxyribonucleotides, which can be incorporated into RNA and DNA. Treatment of cells with 5-FU leads to an accumulation of cells in S-phase and induces p53 dependent apoptosis.	**F6627**	≥99% (TLC)	1 g 5 g 10 g
			47576	BioChemika, ≥99.0% (HPLC)	1 g 5 g 25 g
Ganciclovir		Prodrug nucleoside analog that is phosphorylated following expression of a viral suicide gene encoding thymidine kinase. The phosphorylated active analog is incorporated into the DNA of replicating malignant and normal eukaryotic cells, causing cell death. The cell cycle is irreversibly arrested at the G_2M checkpoint.	**G2536**	≥99% (HPLC)	100 mg

DNA Damage and Repair

DNA Repair Enzymes and Antibodies

Monoclonal Anti-AP Endonuclease antibody produced in mouse

Anti-APE1; Anti-APEN; Anti-APE/Ref1; Anti-APEX1; Anti-APEX nuclease; Anti-AP lysase; Anti-Apurinic/Apyrimidinic Endonuclease; Anti-HAP1

~2 mg/mL, clone APEREF, purified immunoglobulin
Immunogen: recombinant human AP endonuclease.

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Species reactivity: human, dog, rat, mouse

Antigen mol wt ~37 kDa

Application(s):

Immunoblotting........... 0.5-1 µg/mL using total cell extract of Raji cells
Indirect ELISA........suitable
Immunocytochemistry........suitable
Array........suitable
Isotype........IgG1

-20°C DRY ICE

A2105	200 µL

Monoclonal Anti-DNA Ligase I antibody produced in mouse

~1 mg/mL, clone 1A9, purified immunoglobulin
Immunogen: bovine DNA ligase I.

Solution in phosphate buffered saline, pH 7.4, containing 0.08% sodium azide.

Species reactivity: human, bovine

Antigen mol wt ~125 kDa

Application(s):

Immunoblotting........ 1-5 µg/mL using HeLa or DiFi cells
Direct ELISA........suitable
Isotype........IgG1

-20°C WET ICE

D7690	100 µg

Monoclonal Anti-Excision Repair Cross Complementing Protein-1 antibody produced in mouse

Anti-ERCC-1

~1 mg/mL, clone 8F1, purified immunoglobulin
Immunogen: recombinant full length human ERCC1 protein.

Solution in phosphate buffered saline pH 7.4, containing 0.08% sodium azide.

Species reactivity: human, rat

Antigen mol wt 33-36 kDa

Application(s):

Immunoblotting........ 1-5 mg/mL using A431 or HeLa cells
Immunoprecipitation........ 2 µg using 1 mg protein lysate
Immunohistochemistry........ 2-4 µg/mL using human tonsil tissues
Isotype........IgG1

-20°C WET ICE

E8903	100 µg

Methylpurine DNA Glycosylase human

AAG; MPG

MPG is a base excision repair (BER) protein that removes the mutated N-methylpurine nucleotide from alkylated DNA, creating an apurinic/apyrimidinic site. MPG recognizes and excises 3-methyladenine, 7-methylguanine, 3-methylguanine, N-6-ethanoladenine, and hypoxanthine.

≥80% (SDS-PAGE), recombinant, expressed in *Escherichia coli* (as a MBP fusion protein), activity: ≥5000 units/mg protein

Solution in 50% glycerol (w/v) containing 20 mM Tris-HCl, pH 7.4, 1 mM DTT, 1 mM EDTA, 250 mM NaCl, 0.25% CHAPS, 1% protease inhibitor cocktail (v/v) (Cat. No. P8340).

Apparent mol wt ~70 kDa

Unit definition: One unit will cleave 50% of 0.5 pmole of double stranded DNA oligomer substrate containing a hypoxanthine site lesion in 10 min. at 37 °C.

-70°C DRY ICE

M7940	25 µg

NEW Monoclonal Anti-Methylpurine DNA N-Glycosylase antibody produced in mouse

Anti-AAG; Anti-3-Alkyladenine DNA glycosylase; Anti-Alkylpurine DNA N-glycosylase; Anti-APNG; Anti-Methyladenine DNA Glycosylase; Anti-MPG

~2 mg/mL, clone MPG80, purified immunoglobulin
Immunogen: recombinant human MPG protein.

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Species reactivity: human

Antigen mol wt ~32 kDa

Application(s):

Immunoblotting........0.5-1 µg/mL using total cell extract of 293T cells expressing MPG
Indirect ELISA........ suitable
Immunocytochemistry........suitable
Isotype........IgG1

-20°C DRY ICE

M6195	25 µl, 200 µl

Monoclonal Anti-Mismatch Repair Protein 2 antibody produced in mouse

Anti-MSH2

~1 mg/mL, clone 2MSH01, tissue culture supernatant
Immunogen: recombinant human mismatch repair protein 2 (MSH2).

Contains 15 mM sodium azide.

Species reactivity: human

Antigen mol wt 102 kDa

Application(s):

Immunohistochemistry (formalin-fixed, paraffin-embedded sections)........ 1:25
Isotype........IgG1

-20°C WET ICE

M6315	500 µL

Ogg1 Assay Kit

The assay is based on Ogg1 glycosylase activity that recognizes and removes the mutated base (8-oxo-G). The substrate is a 23 oligonucleotide containing 8-oxo-dG at its 11th base, labeled with ^{32}P at its 5′ end, and annealed to its complementary strand (containing dC at the opposite base position to the 8-oxo-dG). Upon cleavage of the substrate by the Ogg1 enzyme, the oligonucleotide strands are run on a denaturing gel and a 10 base fragment (labeled cleavage product) is revealed in addition to the original 23 base oligonucleotide band. The detection is performed by autoradiography.

The Ogg1 Assay Kit:

- Provides a tool for measurement of Ogg1 enzyme activity
- Contains control Ogg1 enzyme enabling screening of Ogg1 modulators
- Can be used with cell lysates and purified enzyme preparation

Sufficient for 180 assays

Components

Ogg1 Substrate 8-oxo-G strand .... 1 vial
Ogg1 Substrate complementary strand.................................. 1 vial
Reaction Buffer .... 10 X 1.5 mL
Stop Solution 1mL
Ogg1 (control enzyme) .... 10 µg
Putrescine ....1 mL
SigmaSpin™ Post-Reaction Purification Columns .... 2 each

CS0710	1 kit

Ogg1 from mouse

8-Oxoguanine-DNA glycosylase

≥90% (SDS-PAGE), recombinant, expressed in *Escherichia coli*, activity: >20,000 units/mg protein

Solution in 50% (w/v) glycerol containing 20 mM Tris, pH 7.5, 200 mM sodium chloride, 1 mM EDTA, and 1 mM DTT.

Apparent mol wt 38.8 kDa (345 amino acid protein)

Unit definition: One unit is the amount of protein that cleaves 50% of 0.5 pmol double-stranded, 8-oxoguanine-mutated DNA oligomer substrate in 10 min at 37 °C.

-20°C WET ICE

O2135	10 µg

Uracil DNA Glycosylase from *Escherichia coli*

DNA Uracil Glycosylase; UDG; Uracil N-glycosylase
[59088-21-0]

UDG catalyzes the removal of uracil residues from both single- and double-stranded DNA, but not RNA. This reaction leaves the DNA sugar-phosphodiester backbone intact. The resulting DNA is not suitable for use as a hybridization target or as a template for DNA polymerases.

Solution in 30 mM Tris-HCl, pH 7.5, 1 mM DTT, 0.05% (w/v) Tween® 20, 1 mM EDTA, 150 mM NaCl, 50% (v/v) glycerol.

Unit definition: One unit catalyzes the release of 1 nmol of free uracil from ^{3}H-poly(dU) in 1 hr at 37°C.

Concentration...1 unit/µL

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-20°C DRY ICE

U1257	100 units

Cytochemistry Kits

Sigma-Aldrich cytochemistry assays are used in the diagnosis of acute myeloid leukemia and in the staining of inflammatory tissue exudates for characterizing neutrophils, lymphocytes and macrophages. The prepackaged kits are a cost effective way to bring testing in-house with no capital equipment requirements.

Classic methods include:

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Antitumor Agents

Cephalomannine

$C_{45}H_{53}NO_{14}$ FW 831.90 [71610-00-9]

Antitumor; antiproliferative. Promotes the formation of highly stable microtubules that resist depolymerization but does not induce cytokine secretion.

≥97% (HPLC)

C4991	1 mg, 5 mg

Chrysomycin A

$C_{28}H_{28}O_9$ FW 508.52 [82196-88-1]

Antibiotic from *Streptomyces* sp. Inhibits the catalytic activity of human topoisomerase II. Exhibits antitumor activity against human cell lines K562, HT29, MCF7, PC6, and MKN28.

≥98% (HPLC)

2-8°C

C6616	100 µg

Chrysomycin B

$C_{27}H_{28}O_9$ FW 496.51 [83852-56-6]

Antibiotic from *Streptomyces* sp. Inhibits the catalytic activity of human topoisomerase II. Exhibits antitumor activity against human cell lines K562, HT29, MCF7, PC6, and MKN28.

≥98% (HPLC)

2-8°C

C6491	100 µg

CIL-102

1-[4-(Furo[2,3-b]quinolin-4-ylamino)phenyl]ethanone
$C_{19}H_{14}N_2O_2$ FW 302.33 [479077-76-4]

Tubulin polymerization inhibitor: apoptosis inducer.

US Patent No. 6,750,223. Sold for non-human research purposes under a non-exclusive license agreement with Kaohsiung Medical University.

≥95% (HPLC)

-20°C WET ICE

C5492	10 mg

17-Dimethylaminoethylamino-17-demethoxy-geldanamycin

17-DMAG
$C_{32}H_{48}N_4O_8$ FW 616.75

17-DMAG is a more potent water soluble analog of geldanamycin. 17-DMAG has shown more antitumor activity than 17-AAG. Inhibits cancer growth and promotes apoptosis in multiple cell lines.

-20°C

D5193	1 mg

Finasteride

N-*tert*-Butyl-3-oxo-4-aza-5α-androst-1-en-17β-carboxamide; N-(2-Methyl-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide; MK-906 $C_{23}H_{36}N_2O_2$ FW 372.54 [98319-26-7]

Selective 5α-reductase inhibitor; antiandrogen.

≥97% (HPLC)

-20°C

F1293	100 mg

ICRF-193

meso-4,4′-(3,2-butanediyl)-bis(2,6-piperazinedione)
$C_{12}H_{18}N_4O_4$ FW 282.30 [21416-68-2]

ICRF-193 induces a G_2 checkpoint that is associated with an ATR dependent inhibition of polo-like kinase 1 (Plk1) activity and a decrease in cyclin B1 phosphorylation. Induces apoptosis in several cell lines including K562 and Molt-4 cells. ICRF-193 is a topoisomerase II inhibitor that targets topoisomerase II-β to a greater extent than it targets topoisomerase II-α and does not cause DNA damage.

≥95%

-20°C

I4659	1 mg, 5 mg

Kazusamycin A

$C_{33}H_{48}O_7$ FW 556.73 [92090-94-3]

Kazusamycin A, an unsaturated branched chain fatty acid with a terminal lactone ring, is a hydroxy analog of Leptomycin B. It has significant *in vitro* cytotoxic activity against various human and mouse tumor lines encompassing a wide range of tissue types. Kazusamycin A exhibits *in vivo* antitumor activity against experimental murine tumors. It inhibits nuclear export and Rev translocation, a regulatory gene product in the HIV genome, at nanomolar concentrations.

from *Streptomyces* sp., ≥95% (HPLC)

70% methanol solution.

-20°C DRY ICE

K1764	1 µg

Linomide

LS-2616; N-Phenylmethyl-1,2-dihydro-4-hydroxyl-1-methyl-2-oxoquinoline-3-carboxamide; Quinoline-3-carboxamide
$C_{18}H_{16}N_2O_3$ FW 308.33 [84088-42-6]

Immunomodulator; antiangiogenic. Upregulates IL-10 and decreases CXC chemokine production. Upregulates mRNA of IL-23, IL-12p35, and IFNγ. Also, antagonizes hepatic effects of TNFα by blocking recruitment of inflammatory cell infiltration.

≥98% (HPLC)

2-8°C

L1793	10 mg

Myoseverin B

2,6-Bis(4-methoxybenzylamino)-9-cyclohexylpurine
$C_{27}H_{32}N_6O_2$ FW 472.58 [361431-27-8]

Tubulin polymerization inhibitor.

≥97% (HPLC)

-20°C DRY ICE

M3316	1 mg, 5 mg

Neocarzinostatin from *Streptomyces carzinostaticus*

Holoneocarzinostatin; NCS; NSC-69856; Zinostatin
[9014-02-2]

Protein-small molecule complex composed of an enediyne chro-mo-phore tightly bound to a 113-amino acid single chain protein. Chemotherapeutic agent which inhibits DNA synthesis, possesses antitumor activity, and induces apoptosis.

Supplied as 100 µg (~9 nmoles) in a solution containing 20 mM MES buffer, pH 5.5.

≥90% (SDS-PAGE), ~0.5 mg/mL

2-8°C WET ICE

N9162	100 µg

Nutlin-3

(±)-4-[4,5-Bis(4-chlorophenyl)-2-(2-isopropoxy-4-methoxyphenyl)-4,5-dihydroimidazole-1-carbonyl]-piperazin-2-one
$C_{30}H_{30}Cl_2N_4O_4$ FW 581.49 [548472-68-0]

Mdm2 (mouse double minute 2) antagonist, p53 pathway activa-tor, apoptosis inducer.

Sold under license from Hoffman-La Roche, Inc. US patent 6,734,302

≥98% (HPLC)

-20°C WET ICE

N6287	1 mg, 5 mg

p53 human

p53 gene is highly conserved and expressed in normal tissues. It is the most commonly mutated gene in human cancer and more than
500 gene mutations have been described in various types of malignancies, hematologic as well as solid tumors. Intact p53 function is essential for the maintenance of the non-tumorogenic phenotype of cells. Thus, p53 plays a vital role in suppressing the development of cancer. Useful for the study of postranslational modification of p53, gel-shift assays, or protein-protein interaction studies.

Supplied at a concentration of 0.2 mg/ml in PBS, pH 7.0.

recombinant, expressed in *Escherichia coli*

>90% (SDS-PAGE)

-20°C WET ICE

P6249	50 µg

>95% (SDS-PAGE)

-20°C WET ICE

P6374	20 µg

PTACH

Cpd 51; S-[6-(4-Phenyl-2-thiazolylcarbamoyl)hexyl] thioisobutyrate
$C_{20}H_{26}N_2O_2S_2$ FW 390.56 [848354-66-5]

HDAC inhibitor; more potent than the majority of HDAC inhibitors except for SAHA.

≥98% (HPLC)

2-8°C

P5874	2 mg

RG108

N-Phthalyl-1-tryptophan
$C_{19}H_{14}N_2O_4$ FW 334.33 [48208-26-0]

DNA methyltransferase (DMNT) inhibitor. Reactivates tumor sup-pressor gene expression (p16, SFRP1, secreted frizzled related protein-1, and TIMP-3) in tumor cells by DNA demethylation. Inhibits human tumor cell line (HCT116, NALM-6) proliferation and increases doubling time in culture.

≥98% (HPLC)

-20°C

R8279	10 mg

SKI II

4-[[4-(4-Chlorophenyl)-2-thiazolyl]amino]phenol
$C_{15}H_{11}ClN_2OS$ FW 302.78 [312636-16-1]

Sphingosine kinase (SK) plays a pivotal role in regulating tumor growth and SK can act as an oncogene. Expression of SK RNA is significantly elevated in a variety of solid tumors, compared with normal tissue from the same patient. A number of novel inhibitors of human SK were identified, and several representative com-pounds were characterized in detail. These compounds demon-strated activity at sub- to micromolar concentrations, making them more potent than any other reported SK inhibitor, and were selec-tive toward SK compared with a panel of human lipid and protein kinases. Kinetic studies revealed that the compounds were not competitive inhibitors of the ATP-binding site of SK. SKI-II inhibitor is orally bioavailable, detected in the blood for at least 8 h, and showed a significant inhibition of tumor growth in mice with IC_{50} = 0.5 µM; SKI II does not act at ATP-binding site. Displays
no inhibition of ERK2, PI 3-kinase, or PKCa at concentrations up to 60 µM. SKI II induces apoptosis and inhibits proliferation in sev-eral other tumor cell lines *in vitro* (IC_{50} = 0.9-4.6 µM).

≥98% (HPLC)

S5696	10 mg, 50 mg

Sulochrin

$C_{17}H_{16}O_7$ FW 332.30 [519-57-3]

Antibiotic from *Aspergillus* and *Penicillium* sp. Fungal metabolite; VEGF inhibitor and antiangiogenic that inhibits the VEGF-induced tube formation of human umbilical vein endothelial cells.

≥98% (HPLC)

2-8°C

S4570	1 mg

New Products

Chemopreventitive Agents

2-Amino-N-quinolin-8-yl-benzenesulfonamide

QBS
$C_{15}H_{13}N_3O_2S$ FW 299.35 [16082-64-7]
Inhibitor of cell cycle at G_2 phase; apoptosis inducer.
≥98% (HPLC)

A3105	5 mg

Borrelidin

Borrelidine; 2-(7-Cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl)-cyclopentanecarboxylic acid; Cyclopentanecarboxylic acid; NSC 216128; Treponemycin
$C_{28}H_{43}NO_6$ FW 489.64 [7184-60-3]
Potent angiogenesis inhibitor that induces apoptosis of the capillary tube-forming cells. Also, displays antimalarial activity against drug-resistant *Plasmodia*. Antimicrobial and selective threonyl t-RNA synthetase inhibitor.
from *Streptomyces parvulus*, ≥98% (HPLC)
-20°C WET ICE

B3061	1 mg

Carnosol

$C_{20}H_{26}O_4$ FW 330.42 [5957-80-2]
A phenolic diterpene with antioxidant and anticarcinogenic activities.
from *Rosemarinus officinalis L.*
-20°C DRY ICE

C9617	5 mg

Etidronate disodium

Dihydrogen (1-hydroxyethylidene)bisphosphonate disodium
$C_2H_6Na_2O_7P_2$ FW 249.99 [7414-83-7]
Bisphosphonate antiresorptive agent. Less potent inhibitor of farnesyl diphosphate synthase (IC_{50} = 80 μM) as compared to the nitrogen containing bisphosphonates
2-8°C WET ICE

P5248	10 mg, 100 mg

Ifosfamide

N,3-Bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide; Ifex
$C_7H_{15}Cl_2N_2O_2P$ FW 261.09 [3778-73-2]
Ifosfamide is a nitrogen mustard compound that is a structural isomer of cyclophosphamide. Ifosfamide is a prodrug that must be transformed by cytochrome P450 to the biologically active component. It is used as an antineoplastic agent in cancer chemotherapy, but ifosfamide is more likely to cause renal toxicity than cyclophosphamide.
≥98%
2-8°C WET ICE

I4909	1 g, 5 g

Lomustine

1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea
$C_9H_{16}ClN_3O_2$ FW 233.70 [13010-47-4]
Antineoplastic agent with cellular DNA effects. Lomustine induces p53 expression in A2870 cells.
≥98%
-20°C

L5918	100 mg

Enzymes

Breast Tumor Kinase Active human

Brk; Protein Tyrosine Kinase 6; PTK6
recombinant, expressed in *Escherichia coli*, ≥85% (SDS-PAGE)
Breast Tumor Kinase (Brk, PTK6) is a member of the non-receptor tyrosine kinases (PTKs). Brk is expressed in breast tumors. Brk promotes cell motility and invasion and functions as a mediator of EGF-induced migration and invasion.
Protein apparent mol wt ~80 kDa
-70°C DRY ICE

B9810	5 μg

Multi-Drug Resistance

Fumitremorgin C

FTC $C_{22}H_{25}N_3O_3$ FW 379.45 [118974-02-0]
from *Neosartorya fischeri*, >98% (HPLC and TLC)
Fumitremorgin C (FTC) is a fungal toxin of the diketopiperazines family of compounds. In mammalian cells, FTC is tremorgenic and causes cell cycle arrest. Fumitremorgin C has been shown to reverse resistance to doxorubicin, mitoxantrane, and topotecan in non-Pgp (P-glycoprotein), non-MRP (multidrug resistance protein) multidrug-resistance (MDR) cells. This reversal of resistance is associated with an increase in drug accumulation. Fumitremorgin C is a specific, selective, and potent inhibitor at micromolar concentrations of the breast cancer resistant protein (BCRP/ABCG2), an ABC transporter associated with chemotherapy resistance. FTC, in combination with mitoxantrone, can be used for the detection of ABCG2 functional activity in several cell lines.
2-8°C WET ICE

F9054	250 μg

Tumor Growth Regulation

(E/Z)-4-HTA monohydrate

(*E*,*Z*)-2-[4-[1-(p-Hydroxyphenyl)-2-phenyl]-1-butenyl]phenoxyacetic acid; 4-Hydroxytamoxifen acid $C_{24}H_{22}O_4 \cdot H_2O$ FW 392.44
Non-steroidal estrogen.
≥90% (HPLC)
2-8°C

H5039	2 mg, 10 mg

PD 173074

N-[2-[4-(Diethylamino)butyl]amino-6- (3,5-dimethoxy-phenyl)pyrido[2,3-d]pyrimidin-7-yl]-N′-(1,1-dimethylethyl)urea
$C_{28}H_{41}N_7O_3$ FW 523.67 [219580-11-7]
Fibroblast growth factor receptor 3 (FGFR3) inhibitor: IC_{50} = 5 nM in inhibition of FGFR3 autophosphorylation; arrested the G_0/G_1 phase of the cell cycle of FGFR3-expressing cells. 100-fold more selective for FGFR3 than for VEGF receptors, IGF-1 receptors, and MAPKs.
≥98% (HPLC)
2-8°C

P2499	5 mg, 25 mg

Reveromycin A

$C_{36}H_{52}O_{11}$ FW 660.79 [134615-37-5]
Polyketide antibiotic from *Streptomyces* sp. Epidermal growth factor (EGF) inhibitor; apoptosis inducer; G_1 phase cell cycle inhibitor having antiproliferative behavior against human cell lines KB and K562 as well as antifungal activity.
≥98% (HPLC)
-20°C WET ICE

R0654	100 μg

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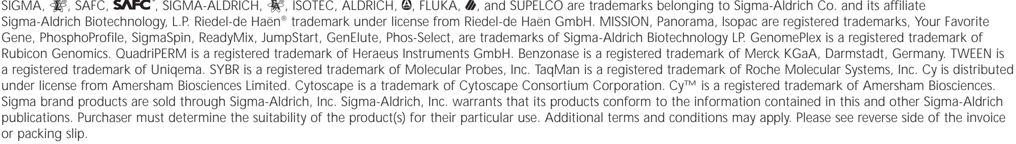

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