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RNA/DNA Stabilization Reagent for Blood/Bone Marrow

Version 05

Content version: October 2013

For simultaneous cell lysis and stabilization of nucleic acids in blood or bone marrow samples

Cat. No. 11 934 317 001
For a total volume of 50 ml of sample material

Store the kit at +15 to +25°C

www.roche-applied-science.com

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1. Product Overview

Contents

⚠ RNA/DNA Stabilization Reagent for Blood/Bone Marrow contains guanidinium isothiocyanate and Triton X-100.
When handling blood, bone marrow, and blood/bone marrow lysates, take the precautions you usually take when handling potentially hazardous material.
Dispose all supernatants properly.

Label	Content
RNA/DNA Stabilization Reagent for Blood/ Bone Marrow	• 1 bottle • 500 ml ready-to-use solution

Product Description

- The RNA/DNA Stabilization Reagent for Blood/Bone Marrow contains guanidinium thiocyanate, Triton X-100, and a reducing chemical.
- After addition to the sample, the reagent does not only result in instantaneous lysis of cells but also effective inactivation of enzymes like ribonucleases, that otherwise degrade RNA.
- Samples, processed by using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow are suitable for the isolation of mRNA or total nucleic acids by, *e.g.*, the mRNA Isolation Kit for Blood/Bone Marrow*.

Application

Cell lysis and simultaneous inactivation of nucleases after addition to blood or bone marrow samples.

Sample Material

- Human peripheral blood, EDTA-, citrate-, or heparin-treated.
- Bone marrow containing EDTA, citrate, or heparin as anti-coagulants.

Sample Volume

The sample or lysate volume, that is compatible with the mRNA Isolation Kit for Blood/Bone Marrow ranges from ≤1.5 to 5 ml of blood or bone marrow corresponding to ≤16.5 ml to 55 ml of lysate.

Number of Tests

10 volumes of RNA/DNA Stabilization Reagent for Blood/Bone Marrow are added to one volume of sample.
The content of one package size is sufficient for stabilization of a total of 50 ml of sample material.

Quality Control

The reagent is function-tested in the following model system:
Detection of tyrosinase mRNA by RT-PCR after addition of MelJu cell mRNA to fresh normal human blood stabilized with the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. The mRNA is purified from the stabilized lysate with the mRNA Isolation Kit for Blood/Bone Marrow.
Recovery of MelJu mRNA is shown by detection of tyrosinase mRNA by RT-PCR.

Storage and Stability

The unopened reagent is stable at +15 to +25°C until the expiration date printed on the label.

Advantages

- **Omission of cell separation prior to mRNA isolation:** mRNA and total nucleic acids can be isolated from whole blood or bone marrow lysates without the risk for loss of fragile cells (*e.g.*, circulating tumor cells) during erythrocyte depletion.
- **Easy stabilization and stable storage after sample taking:** blood or bone marrow is stabilized by simply mixing the reagent and sample. Lysates can be stored for 12 months at −15 to −25°C, or for one day at +2 to +8°C.
- Option of combining the stabilization procedure with the powerful mRNA isolation procedure of the mRNA Isolation Kit for Blood/Bone Marrow.

2. Background Information

RNA Instability

- The extreme instability of RNA is mainly due to the ubiquitous presence of enzymes (RNases) which degrade RNA and can recover activity even after many forms of treatment such as boiling (1).
- **Disrupt cells and stabilize RNA** (**by inactivation of RNases**) **as soon as possible after sample collection.** To obtain good preparations of eukaryotic mRNA, it is necessary to minimize the activity of RNases liberated during cell lysis by using methods that disrupt cells and inactivate RNases simultaneously (1).
- **Do not store blood or bone marrow samples for more than a few hours, if stabilization is not possible**. Even in their natural environment within the cell, most mRNAs are extremely unstable. The storage of cells in an "artifical" environment results in qualitative and quantitative changes of the mRNA content of the cells.

RNA Stabilization

- Disrupting cells in guanidinium lysis buffers is an efficient way to protect against RNase-mediated degradation (2, 3).
- RNA lysis buffers that contain guanidinium thiocyanate or guanidinium-HCl consistently yield a high quality sample (3).

Rare Cell Detection

RT-PCR allows in your research studies the detection of disseminated tumor cells in blood or bone marrow with unprecedented sensitivity (4, 5). Nevertheless some aspects particularly concerning sample stabilization and other steps preceeding the actual mRNA isolation procedure should be considered carefully:

- **Omit cell separation steps** to proactively avoid loss of target cell, whenever possible. Particularily tumor cells and micrometastases are heterogeneous. There is no one tumor and the behaviour of tumor cells or micrometastasis during cell separation is clearly not predictable.
- **Use mRNA instead of total RNA**. For ultra-sensitive detection of rare cells by RT-PCR, the mRNA isolated from a large sample volume (*e.g.*, 1 ml or even more) should be used for one single RT-PCR reaction. 1 ml of normal human blood contains 4 to 20 µg total RNA; too much to be used in one RT-PCR reaction. In case of mRNA the corresponding amount (100 to 400 ng) does not result in decreased sensitivity.
- **Use enough mRNA for detection in one RT-PCR reaction.** Assuming that one tumor cell containing 10 target transcripts has to be detected in the presence of 10^7 white blood cells (2 ml of blood), it is necessary to use at least the mRNA of 2 ml of blood in one single RT-PCR reaction.
- **Be sure that the RNA isolation method efficiently purifies nucleic acids away from inhibitors of the RT-PCR reaction**. Blood and bone marrow contain lots of potent inhibitors of the RT-PCR reaction like hemoglobin and the anti-coagulant heparin. Especially, if the isolated RNA is not diluted for RT-PCR, the efficiency of the purification procedure is of extreme importance.

3. Procedures and Required Material

Additional Equipment Required

- Equipment required for the measurement of reagent and blood volumes
- Bottles or tubes (*e.g.,* Falcon tubes) suitable for mixing RNA/DNA Stabilization Reagent for Blood/Bone Marrow and sample material.
- Dry ice, if sample lysates have to be transported.

Sample Material

Blood and bone marrow samples. Commonly used anti-coagulants like heparin, EDTA, or citrate do not interfere with sample stabilization, mRNA isolation procedure using the mRNA Isolation Kit for Blood/Bone Marrow, or RT-PCR after mRNA purification.

Lysates are only suitable, if stored properly.

Storage conditions:

- not longer than12 months at −15 to −25°C,
- not longer than one day at +2 to +8°C,
- not longer than 6 hours at +15 to +25°C.

Pre-Treatment of the Reagent

The reagent crystallizes at temperatures below +20°C. Check the solution for the absence of crystals before use, and warm to +37°C if it is not completely dissolved. Thoroughly mix before use.

Procedure

Please refer to the following table

Step	Action
❶	Label an appropriate container (bottle or tube) with all important information (*e.g.*, information regarding the identification; date/exact time of collection; delay between collection and stabilization; kind of sample; sample volume, *etc.*).
❷	Transfer the 10 volumes of the RNA/DNA Stabilization Reagent for Blood/Bone Marrow into the container per volume of sample (*e.g.*, 50 ml per 5 ml blood or bone marrow).
❸	Add blood or bone marrow sample and mix vigorously, for example, by vortexing.
❹	Ensure that the sample is properly stored until mRNA and or DNA isolation (storage conditions: not longer than 12 months at −15 to −25°C, not longer than one day at +2 to +8°C, not longer than 6 hours at +15 to +25°C). For transport, deep freezing the lysate is recommended.

Pre-Treatment of Samples Before mRNA Isolation

- If lysates are deep-frozen, thaw samples carefully.
- Prewarm lysate to +15 to +25°C . Thoroughly mix (*e.g.,* by vortexing) to ensure that crystallized material is fully dissolved.
- Do not store the thawed samples at temperatures above +25°C. Pay attention to the storage conditions detailed above.

Recommended Following Isolation

The succeeding mRNA isolation by using the mRNA Isolation Kit for Blood/Bone Marrow includes the following steps:

- Magnetic glass particles (MGPs) are added to a blood or bone marrow lysate, and total nucleic acids (RNA, DNA) are bound onto the MGPs during incubation.
- MGPs are separated by centrifugation or magnetic force and unbound material is removed by washing.
- Nucleic acids are eluted from the MGPs. At this stage, part of the nucleic acids can be used for DNA analysis.
- mRNA is captured from total nucleic acids by using biotin-labeled oligo(dT) and streptavidin-coated magnetic particles (SMPs).
- SMPs are separated by magnetic force and unbound material is removed by washing.
- mRNA is eluted after removal of other nucleic acids (DNA, rRNA, tRNA) by washing.

4. Typical Results

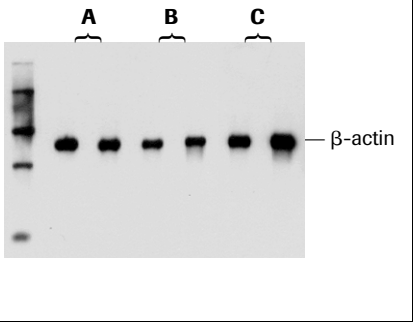
Introduction

The following figures show typical results regarding:

- Stability of sample lysates
- detection of melanoma cells in bone marrow (model system).

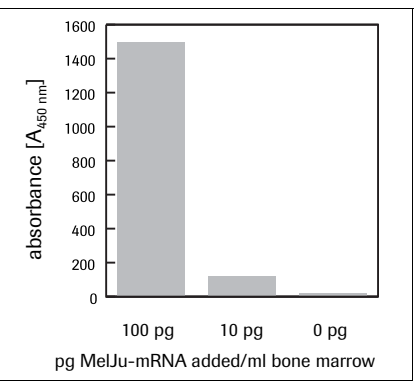
mRNA is Isolated, Maintaining a High Degree of Integrity

Fig. 1: A total of 6 ml of normal human heparinized blood was lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. Three aliquots of the lysate corresponding to 2.0 ml blood each were stored at +2 to +8°C (A), +15 to +25°C (B), and −15 to −25°C (C) for 4 days. The mRNA was isolated in duplicate from each sample using the mRNA Isolation Kit for Blood/Bone Marrow. Eluates corresponding to 1 ml of blood were analyzed by Northern blotting using a DIG-labeled anti-sense RNA β-actin probe to confirm integrity of the mRNA.



Bone Marrow is Suitable for Tumor Cell Detection

Fig. 2: 3 ml of human heparinized bone marrow were lysed using the RNA/DNA Stabilization Reagent for Blood/Bone Marrow. Three aliquots of the lysate corresponding to 1 ml bone marrow each, were spiked with 100 pg, 10 pg, or 1 pg of melanoma cell line (MelJu) mRNA. The mRNA was isolated from each lysate using the mRNA Isolation Kit for Blood/Bone Marrow. The eluate was subjected to RT PCR using tyrosinase cDNA specific primers (HTYR1, HTYR2). The PCR product was detected by PCR ELISA.



References

1 Sambrook J., Fritsch E. F., & Maniatis T. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

2 Chirgwin J. M. *et al.* (1979) *Biochemistry* **18,** 5294.

3 Farrell R. E. (1993) RNA Methodologies: A Laboratory Guide for Isolation and Characterization, Academic Press, San Diego.

4 Smith *et al.* (1991) *Lancet* **338,** 1227.

5 Moreno *et al.* (1992) *Cancer Res.* **52,** 6110.

5. Troubleshooting

Problem	Possible Cause	Recommendation
Low yield of mRNA (*e.g.*,< 50 ng/ml of human blood after isolation using the mRNA Isolation Kit for Blood/Bone Marrow).	Lysate has not been stored properly before isolation of mRNA	Stability of lysates: • not longer than 12 months at −15 to −25°C, • not longer than one day at +2 to +8°C, • not longer than 6 hours at +15 to +25°C. Store lysates accordingly.
	Blood has not been stored properly before stabilization	Storage of whole blood results in continuous loss of mRNA. Do not store unstabilized samples for longer than a few hours.
Integrity of isolated mRNA is not appropriate	Lysate has not been stored properly before isolation of mRNA	Stability of lysates: • not longer than 12 months at −15 to −25°C, • not longer than one day at +2 to +8°C, • not longer than 6 hours at +15 to +25°C. Store lysates accordingly.

6. Supplementary Information

6.1 Conventions

Text Conventions To make information consistent and easier to understand, the following text conventions are used in this document:

Text Convention	Use
Numbered instructions labeled ❶, ❷ *etc.*	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

6.2 Changes to Previous Version

- Regulatory Disclaimer updated
- Editorial changes

6.3 Ordering Information

Kits

Product	Pack size	Cat. No.
mRNA Isolation Kit for Blood/Bone Marrow	100 (30) isolations from 1.5 ml (5 ml) of sample material	11 934 333 001
mRNA Capture Kit	1 kit (192 reactions)	11 787 896 001
mRNA Isolation Kit	1 kit for the isolation of at least 70 µg poly(A^+) RNA	11 741 985 001
DNA Isolation Kit for Mammalian Blood	1 kit for up to 10 ml mammalian blood	11 667 327 001

Single reagents

Product	Pack size	Cat. No.
Red Blood Cell Lysis Buffer	100 ml for 50 to 500 reactions	11 814 389 001
Tth DNA Polymerase	2× 250 U	11 480 022 001
Titan One Tube RT-PCR System	100 reactions	11 855 476 001
Reverse Transcriptase M-MuLV	500 U	11 062 603 001
Reverse Transcriptase AMV	1,000 U	10 109 118 001
First strand cDNA Synthesis Kit for RT-PCR (AMV)	1 kit	11 483 188 001
cDNA Synthesis System	1 kit	11 117 831 001
Expand Reverse Transcriptase	1,000 U 5,000 U	11 785 826 001 11 785 834 001
5´/3´ RACE Kit, 2nd Generation	1 kit	03 353 621 001
Titan One Tube RT-PCR Kit	50 reactions	11 939 823 001

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