

DETECTION AND PURIFICATION

HIS-Select™

A Highly Selective Chemistry for His-tagged Protein Purification

Sigma's HIS-Select products have the ability to purify His-tagged proteins quickly and with high selectivity. This is made possible by the **non-charged** linkage chemistry that attaches the chelate to the agarose bead matrix. Most other immobilized metal affinity chromatography (IMAC) systems for His-tagged protein purification utilize a charged chelate linkage. Extraneous charges on the resin will attract any oppositely charged amino acid in a protein, thereby increasing non-specific binding. In addition, because the HIS-Select linker is **hydrophilic**, like the surface of most proteins, there is no interaction between the resin and non-specific proteins due to polarity of the resin.

The **highly pure tetradentate** chelate feature of the resin also aids in reducing non-specific binding and increasing binding capacity. Tetradentate chelates hold the metal ion at four coordination points opposed to three points like IDA type chelates. This makes the tetradentate chelate preferred over IDA type chelates because the metal ion is held tightly and this results in less metal leaching from the affinity gel.

These technologies combined, the non-charged, hydrophilic linker and the highly pure tetradentate chelate, allow HIS-Select to provide **superior selectivity and binding capacity** for your His-tagged protein. Thus, HIS-Select allows you to eliminate time-consuming secondary purification and allows for true **one-step purification**.

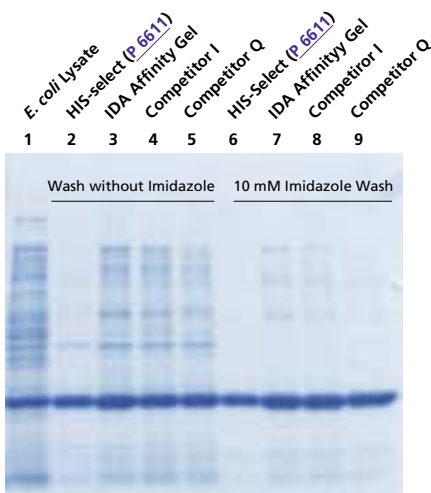


Figure 1. Less Imidazole required than Competition

Lysates from *E. coli* containing 8 mg HIS-tagged protein per ml resin were loaded onto the resins in the presence of either no or 10 mM imidazole. These were incubated for 30 minutes at room temperature while rotating. Resins were washed three times with wash buffer (50 mM NaPO₄, 300 mM NaCl, pH 8) and the His-tagged protein was eluted with 250 mM imidazole. Eluted protein was run on a gel and stained with EZBlue stain ([G_1041](#)).

Features & Benefits

- Highly selective for higher purity
- Non-charged, hydrophilic linkage reduces non-specific binding
- Highly pure tetradentate chelate for higher binding capacity
- One-step purification

HIS-Select Requires Less Imidazole

Imidazole has traditionally been added to the wash buffer to modulate non-specific binding when purifying His-tagged proteins with affinity gels. However, the HIS-Select technology is so selective that it requires much less **imidazole (0-10 mM)** in the wash buffer than has traditionally been used (Figure 1). Even with no imidazole in the wash buffer, the data clearly indicates that little non-specific binding occurs when using the HIS-Select Affinity Gel in lane 2 in comparison to the lanes 3-5. Furthermore, washing with the low concentration of 10 mM imidazole, non-specific binding has been virtually eliminated (lane 6) with the HIS-Select Affinity Gel. Using concentrations of imidazole higher than 10 mM will not further decrease non-specific binding.

Figure 1: One Step Purification



1 Low Range Sigma Marker ([M_3913](#))
 2 Whole Cell Extract in Sample Buffer
 3 Whole Cell Extract with CellLytic B™
 4 Flow Through
 5 Wash 1
 6 Wash 2
 7 Elute 1
 8 Elute 2
 9 Elute 3
 10 Elute 4
 11 Low Molecular Weight Sigma Marker ([M_3913](#))

HIS-Select™ Nickel Affinity Gel

This is the tool of choice for small to medium scale purification of HIS-tagged proteins. Figure 1 shows a typical purification using HIS-Select Nickel Affinity Gel. Note that in elution lanes 7-10 there is virtually no non-specific binding.

Product Code	Description	Size
P_6611	HIS-Select Nickel Affinity Gel	5 mL
		25 mL
		100 mL
		500 mL

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HIS-Select™ HF Nickel Affinity Gel

HIS-Select HF (High Flow-Rate) Nickel Affinity Gel brings the superior selectivity of HIS-Select technology to a highly cross-linked agarose designed for higher flow rates and mechanical stability under pressure. This product is designed for production scale purification and FPLC™ applications.

Product Code	Description	Size
H 0537	HIS-Select HF Nickel Affinity Gel	10 mL 25 mL 100 mL 500 mL



Selectivity:

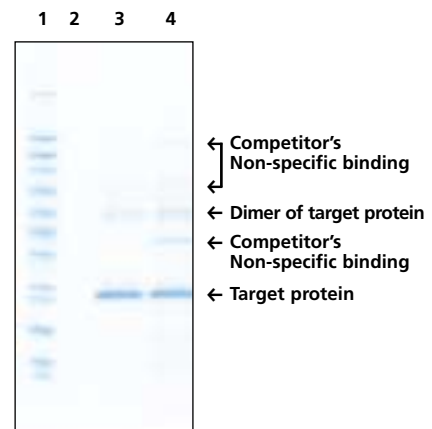
HIS-Select vs. Leading Competitor

E. coli lysates containing a HIS-tagged protein were purified using standard procedures. Proteins were bound at pH 8 in 50 mM sodium phosphate, 300 mM sodium chloride buffer containing 10 mM imidazole. Columns were washed with buffer containing 10 mM imidazole and eluted with buffer containing 250 mM imidazole.

NEW HIS-Select™ Cobalt Affinity Gel

For those who prefer to use cobalt as the affinity metal ion for HIS-tagged protein purification, our new HIS-Select Cobalt Affinity Gel allows for high purity, low non-specific binding and high binding capacity for your protein. HIS-Select Cobalt Affinity Gel also works well for purification of HIS-tagged proteins in native, denaturing, or mild reducing conditions.

Product Code	Description	Size
H 8162	HIS-Select Cobalt Affinity Gel	5 mL 25 mL 100 mL



Lane 1 – Sigma Wide Range Marker (M 4038)

Lane 2 – Empty

Lane 3 – HIS-Select Cobalt Affinity Gel ([H 8162](#)) Elution

Lane 4 – Competitor C Cobalt Metal Affinity Resin Elution

E. coli cell extract containing a HIS-tagged protein was purified using HIS-Select Cobalt Affinity Gel ([H 8162](#)) and a leading competitor's Cobalt Metal Affinity Resin using standard protocols for each. Samples were run on a 4-20% Tris-Glycine gel and stained with EZ-Blue Gel Staining Reagent ([G 1041](#)).

EZview™ Red HIS-Select™ Nickel Affinity Gel

When performing small-scale affinity capture, such as molecular pull-down, the affinity matrix is difficult to see in the microcentrifuge tube with standard resins. Accidental aspiration of the resin leads to quantitative variability in results. The EZview Red Affinity Gel greatly reduces the risk of pellet loss. EZview resins perform as well as conventional non-colored affinity gels, but allow the user to easily differentiate pellet from supernatant. This correlates to more accurate data because less target protein is lost.

Direct Affinity Capture of Tagged Proteins.

Target proteins were spiked (+), or not spiked (-), into COS-7 cell lysates and captured using either standard resin or EZview HIS-Select HC Nickel affinity beads. Western blots of 12% SDS-PAGE gels are shown. Blots were probed with anti-HAT™ tag antibody plus alkaline phosphatase conjugated secondary antibody and developed with BCIP/NBT.

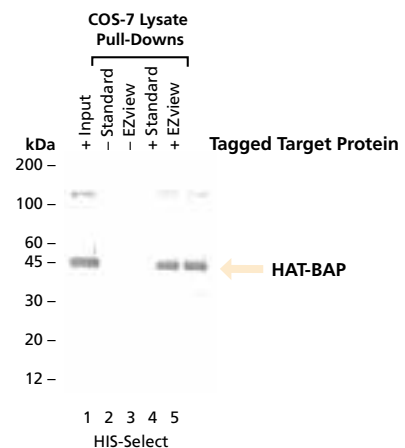
Results:

Lane 1: Control target protein

Lanes 2 & 3: EZview and standard affinity beads show no detectable background

Lanes 4 & 5: EZview and standard affinity beads show equal binding capacity

Product Code	Description	Size
E 3528	EZview Red HIS-Select Nickel Affinity Gel	1 mL 5 x 1 mL

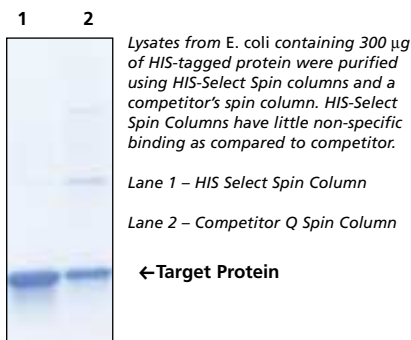


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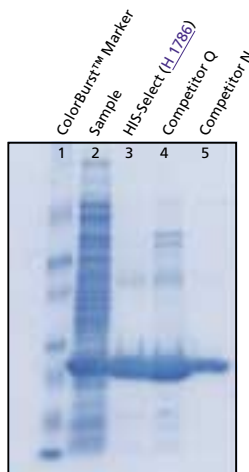


Selectivity: HIS-Select Cartridge vs. Leading Competitors

E. coli lysates containing a HIS-tagged protein were purified using standard procedures. Cartridge was run using a BioLogic™ chromatography system from BioRad. Five milliliters of lysate were loaded to the cartridges and proteins from the lysate were bound at pH 8.0 in 50 mM sodium phosphate, 300 mM sodium chloride buffer containing 10 mM imidazole. The cartridges were washed with buffer containing 10 mM imidazole and eluted with the buffer above plus 250 mM imidazole.



Less Non-specific Binding and Higher Binding Capacity



MAT tagged target protein was mixed with 50 µl of each magnetic bead. The beads were washed, and the target protein was eluted with 250 µl of buffer containing 250 mM imidazole. Elutions were mixed 1:1 with sample buffer and 10 µl of each elution were loaded onto the gel. Sigma's HIS-Select Magnetic Agarose Beads (H 1786) show high binding capacity and greater specificity compared to the competitors.

HIS-Select™ Nickel Cartridges

HIS-Select Cartridges are conveniently pre-packed columns with 1.25 ml of HIS-Select Nickel Affinity Gel. They allow for rapid, small-scale purification of HIS-tagged proteins. Columns contain fittings for syringe purification and connection to FPLC™ or other medium pressure systems.

Product Code	Description	Size
H 8286	HIS-Select Nickel Cartridges	5 ea.

NEW HIS-Select™ Nickel Spin Columns

Pre-packed and ready-to-use HIS-Select Spin Columns allow for fast and consistent small-scale purification of HIS-tagged protein from crude cell extracts. The Spin Columns contain a matrix of silica particles and use the HIS-Select technology to attain high protein purity and low non-specific binding. Spin Columns allow for fast purification in approximately 15 minutes!

Product Code	Description	Size
H 7787	HIS-Select Nickel Spin Columns	10 ea. 50 ea.

NEW HIS Select™ Nickel Magnetic Beads

The HIS-Select Magnetic Agarose Beads are designed for use in automated and small-scale affinity capture (molecular pull-down) purification of HIS-tagged proteins. The magnetic properties make manipulations such as repetitive washings and recovery of bound protein from the beads quicker and easier. This leads to greater speed, experimental reproducibility, and more accurate quantification of your protein of interest. HIS-Select Magnetic Beads are supplied in a 50% suspension in 30% ethanol. A magnetic separator ([M 1167](#)) for 1.5 ml microcentrifuge tubes is also available.

Product Code	Description	Size
H 1786	HIS-Select Nickel Magnetic Beads	2 x 1 mL 5 x 1 mL

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NEW HIS-Select™ Nickel Filter Plate

Filter plates incorporate the speed, consistency and convenience of spin columns into a multi-sample format. This pre-packed 96-sample plate is useful for small-scale histidine-tagged protein purification from crude cell extracts in less than 15 minutes. Filter plates are also compatible with centrifugation, vacuum-manifold and robotics systems. 96 Deepwell 2 ml collection plates ([P 7616](#) or [P 1492](#)) are also available for use with HIS-Select Filter plates.



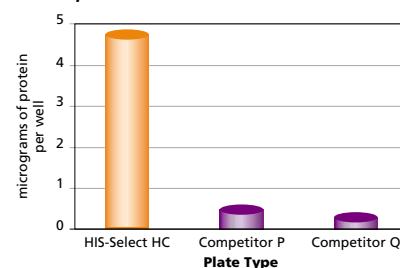
Product Code	Description	Size
H 0413	HIS-Select Filter Plate	1 ea. 5 x 1 ea.

HIS-Select™ HC Nickel-Coated 96-Well Plates

HIS-Select High Capacity (HC) Nickel Coated Plates are coated with a proprietary, high-density nickel chelate matrix. This patent-pending coating allows for greater per-well binding capacity than any other commercial histidine-binding plates and low non-specific binding. The 96-well plates can capture $\geq 4 \mu\text{g}$ protein per well and the 384 well plates capture $\geq 2 \mu\text{g}$ protein per well.

Product Code	Description	Size
S 5563	HIS-Select HC Nickel-Coated 96-Well Plates	1 ea. 5 ea.
H 1661	HIS-Select HC Nickel-Coated 384-Well Plates	1 ea. 5 x 1 ea.

HIS-Select HC Plates bind more protein than the competition

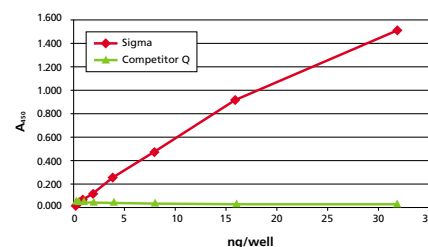


20 μg of pure His-tagged protein was added to the wells of the HIS-Select HC 96-well Plate, and two Competitor Plates for capture of target protein. All plates were incubated for 4 hours at room temperature. The protein was eluted with 250 mM imidazole and quantified with the Bicinchoninic Acid Kit for Protein Determination Kit ([BCA-1](#)).

HIS-Select™ HS Nickel-Coated 96-Well Plates

HIS-Select High Sensitivity (HS) Nickel Coated Plates are designed for highly accurate low-level detection of HIS-tagged proteins. The captured proteins can be detected using standard enzyme-linked assay (ELISA) techniques. These plates are pretreated to reduce non-specific binding and are sensitive enough to capture as little as 1 ng/well of HIS-tagged protein.

Product Code	Description	Size
S 5688	HIS-Select HS Nickel-Coated 96-Well Plates	1 ea. 5 ea.



HIS-Select HS Nickel Coated 96-well plate comparison with a competitor's plate for capture of HAT-BAP target protein from cell lysates.

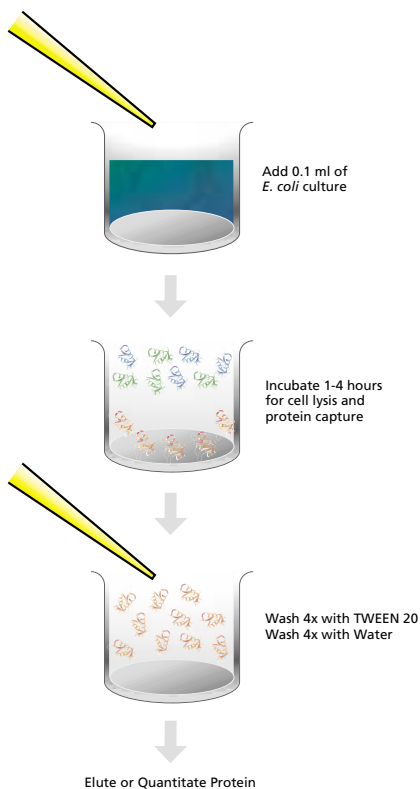
Imidazole

For Molecular Biology, minimum 99%. Wash and elution buffer for HIS-tagged protein purification with HIS-Select affinity gels, magnetic beads, cartridges, spin columns and plates.

DNase, RNase, proteasenone detected

Product Code	Description	Size
I 5513	Imidazole	5 g 25 g 100 g

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NEW HIS-Select™ iLAP™ Plates

Integrated Lysis and Affinity Purification Plates

Sigma's HIS-Select iLAP 96-well Plates are coated with both a cell lysis reagent and the HIS-Select nickel chelate matrix. This patent-pending technology allows for cells to be lysed and the His-tagged protein to be captured all in one step. Cell lysis and protein extraction is highly efficient and eliminates the need to harvest cells from the culture. The HIS-Select coating is highly selective for His-tagged proteins, reduces non-specific binding, and has a high binding capacity of $\geq 4 \mu\text{g}$ protein/well. This makes these plates ideal for rapid colony screening and protein:protein interaction assays.

Features & Benefits

- One-step cell lysis and His-tagged protein purification
- Efficient cell lysis without harvesting cells from the culture
- HIS-Select— highly selective for His-tagged proteins, less non-specific binding, and high binding capacity
- Ideal for rapid colony screening

Product Code	Description	Size
H 9412	HIS-Select iLAP Plates	1 ea. 5 x 1 ea.

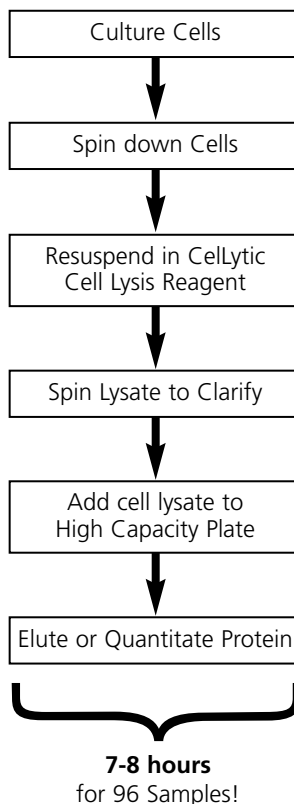
One-step Cell Lysis and Purification



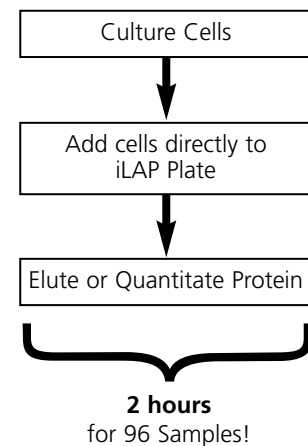
100 μl of *E. coli* culture expressing HIS-tagged protein was added to each well of the iLAP plate. Plate was incubated at room temperature for 2 hours to lyse the cells and capture the protein. Plate was washed and protein from one random well was eluted with 250 mM imidazole for analysis. Gel was stained with ProteoSilver™ Silver Stain Kit (PROT-SIL1).

Lane 1: *E. coli* culture lysate
Lane 2: Eluted protein

Traditional Procedure



Procedure with iLAP Plates



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HIS-Select™ M Affinity Capture Kit

This kit provides all the components needed for the extraction and rapid small-scale affinity capture of HIS-tagged proteins from mammalian cells. The EZview™ Red HIS-Select Nickel Affinity Gel provided in the kit exhibits high selectivity of HIS-tagged proteins and the very low non-specific binding of other proteins. Plus the red color allows for less pellet loss during aspiration when performing immunoprecipitation or molecular-pull down experiments. The Cellytic™ M component is a quick and effective lysis buffer for both adherent and suspension cells that express the HIS-tagged protein. The kit is sufficient for 50 affinity capture purifications and a highly detailed protocol for affinity capture is provided.

Product Code	Description	Size
EHM-1	HIS-Select M Affinity Capture Kit	1 kit

Components

Cellytic M
5x Phosphate Buffer (250 mM Sodium Phosphate pH 8, 1.5 M NaCl)
2.5 M Imidazole
EZview Red HIS-Select HC Nickel Affinity Gel
5% TWEEN® 20
2.5% Hexadecyltrimethylammonium Bromide (CTAB)
Protease Inhibitor Cocktail

HIS-Select™ M Purification Kit

This kit provides all the components needed for the extraction and rapid affinity purification of HIS-tagged proteins from mammalian cells. The HIS-Select Nickel Affinity Gel provided in the kit exhibits high selectivity of HIS-tagged proteins and the very low non-specific binding of other proteins. The Affinity Gel allows for one-step purification of at least 15 mg of an approximately 30 kDa HIS-tagged protein per ml of affinity gel. The Cellytic™ M component is a quick and effective lysis buffer for both adherent and suspension cells that express the histidine-tagged protein. The kit is sufficient for 5 x 1 ml affinity purification columns and a highly detailed protocol for affinity purification is provided.

Product Code	Description	Size
HMP-1	HIS-Select M Purification Kit	1 kit

Components

Cellytic M
5x Phosphate Buffer (250 mM Sodium Phosphate pH 8, 1.5 M NaCl)
2.5 M Imidazole
HIS-Select Nickel Affinity Gel
5% TWEEN® 20
2.5% Hexadecyltrimethylammonium Bromide (CTAB)
Polypropylene chromatography columns

HIS-Select™ Y Purification Kit

This kit provides all the components needed for the extraction and rapid affinity purification of HIS-tagged proteins from yeast cells. The HIS-Select Nickel Affinity Gel provided in the kit exhibits high selectivity of HIS-tagged proteins and the very low non-specific binding of other proteins. The Affinity Gel allows for one-step purification of at least 15 mg of an approximately 30 kDa HIS-tagged protein per ml of affinity gel. The Cellytic™ Y component is a quick and effective lysis buffer for yeast cells that express the HIS-tagged protein. The kit is sufficient for 5 x 1 ml affinity purification columns and a highly detailed protocol for affinity purification is provided.

Product Code	Description	Size
HYP-1	HIS-Select Y Purification Kit	1 kit

Components

Cellytic Y
5x Phosphate Buffer (250 mM Sodium Phosphate pH 8, 1.5 M NaCl)
2.5 M Imidazole
HIS-Select Nickel Affinity Gel
5% TWEEN® 20
2.5% Hexadecyltrimethylammonium Bromide (CTAB)
Polypropylene chromatography columns

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HIS-Select™ Quick Reference Guide



Nickel Affinity Gel

High Flow (HF)
Nickel Affinity Gel

Cobalt Affinity Gel

Nickel Cartridges

Nickel Spin Columns

Product Number	P 6611	H 0537	H 8162	H 8286	H 7787
Package Sizes	5 ml, 25 ml, 100 ml, 500 ml	10 ml, 25 ml, 100 ml, 500 ml	5 ml, 25 ml, 100 ml	5 ea	10 ea, 50 ea
Application	<ul style="list-style-type: none"> • Gravity Flow Column • Small-Med. Scale 	<ul style="list-style-type: none"> • FPLC™ • Production Scale 	<ul style="list-style-type: none"> • Gravity Flow Column • Small-Med. Scale 	<ul style="list-style-type: none"> • FPLC • Syringe Purification • Small Scale 	<ul style="list-style-type: none"> • Mini Prep • Process by Centrifugation or Vacuum
Scale per Sample	100 µg – 10 g	100 µg – 100 g	100 µg – 10 g	15 – 20 mg	<ul style="list-style-type: none"> • 150 µg • 600 µl/load
Binding Capacity	15 mg/ml	15 mg/ml	15 mg/ml	15 mg/cartridge	150 µg/column
Matrix	6% Beaded Agarose	6% Beaded Agarose, Highly Cross-linked	6% Beaded Agarose	6% Beaded Agarose	Silica Particles (Porous, Spherical)
Bead Size	45 – 165 µm	45 – 165 µm	45 – 165 µm	45 – 165 µm	Approx. 20 µm
Exclusion Limit (MW) or Pore Size	4 x 10 ⁶ Da	4 x 10 ⁶ Da	4 x 10 ⁶ Da	4 x 10 ⁶ Da	0.1 µm (approx. 10 x 10 ⁶ Da)
Max. Linear Flow Rate (Max. pressure)	150 cm/hr (5 psi)	3000 cm/hr (200 psi)	150 cm/hr (5 psi)	150 cm/hr (5 psi)	N/A
Recommended Flow Rate for 1 x 2 cm Column	1 ml/min	5 ml/min	1 ml/min	1 ml/min	N/A
Recommended Binding Time/Speed	N/A	N/A	N/A	N/A	Equivalent to approx. 80 x g Centrifugation
Optimal pH Stability	3-10	3-10	3-10	3-10	3-10
Physical Form	50% suspension in 30% ethanol	50% suspension in 30% ethanol	50% suspension in 30% ethanol	1.25 ml pre-packed cartridge	Pre-packed dry matrix in spin column
Antimicrobial Agent	30% ethanol	30% ethanol	30% ethanol	30% ethanol	N/A
Storage	2-8 °C	2-8 °C	2-8 °C	2-8 °C	2-8 °C
Recommended Imidazole Conc. for Load/Wash	0-10 mM	0-10 mM	0-10 mM	0-10 mM	0-5 mM
Recommended Imidazole Conc. for Elution	250 mM	250 mM	250 mM	250 mM	250 mM

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EZview™ Red Affinity Gel	Nickel Magnetic Beads	High Sensitivity (HS) Nickel Coated Strip Plates	High Capacity (HC) Nickel Coated Plates 96-well 384-well	iLAP™ High Capacity (HC) Nickel Coated Plates 96-well	Nickel Filter Plates 96-well
E 3528	H 1786	S 5688	S 5563 , H 1661	H 9412	Inquire
1 ml, 5 x 1 ml	2 x 1 ml, 5 x 1 ml	1 ea, 5 ea	1 ea, 5 x 1 ea	1 ea, 5 x 1 ea	1 ea, 5 ea
<ul style="list-style-type: none"> • Mini Prep Pull-Downs 	<ul style="list-style-type: none"> • Rapid Mini Prep Magnetic Pull-Downs • High Throughput Screening • Compatible with Robotics when used in 96-well plates 	<ul style="list-style-type: none"> • High Throughput Screening (ELISA) • Compatible with Robotics 	<ul style="list-style-type: none"> • High Throughput, High Capacity Screening • Multi-Sample Mini Prep • In-Well Protein Assays • Compatible with Robotics 	<ul style="list-style-type: none"> • Direct Cell Lysis and Purification of Target Protein in Well • High Throughput, High Capacity Screening • Multi-Sample Mini Prep • In-Well Protein Assays • Compatible with Robotics 	<ul style="list-style-type: none"> • Small Scale • High Throughput Screening • Process by Centrifugation or Vacuum • Compatible with Robotics
10 – 500 µg per pull-down	10 µg – 15 mg per pull-down	<ul style="list-style-type: none"> • 1 ng – 1 µg/well • 96-well: 200 µl/well 	<ul style="list-style-type: none"> • 1 – 4 µg/well • 96-well: 200 µl/well • 384-well: 60 µl/well 	<ul style="list-style-type: none"> • 1 – 4 µg/well • 96-well: 200 µl/well 	<ul style="list-style-type: none"> • 1 mg/well • 1 ml/load
15 mg/ml	15 mg/ml	Sensitivity: ≤1 ng/well	96-well: ≥4 µg/well 384-well: ≥2 µg/well	≥4 µg/well	1 mg/well
6% Beaded Agarose	Paramagnetic Iron Impregnated 6% Beaded Agarose	Clear polystyrene plate; Proprietary Coating	Clear Polystyrene Plate; Proprietary Coating	Clear polystyrene plate; Proprietary Coatings for lysis and purification	Silica Particles (Porous, Spherical)
45 – 165 µm	20 – 75 µm	N/A	N/A	N/A	Approx. 20 µm
4 x 10 ⁶ Da	4 x 10 ⁶ Da	N/A	At least 300 kDa	At least 300 kDa	0.1 µm (approx. 10 x 10 ⁶ Da)
N/A	N/A	N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A	N/A	N/A
1 hr at 4 °C	0.5 hr at 25 °C	1 hr at 25 °C	96-well: 4 hr at 25 °C 384-well: 2 hr at 25 °C	1-4 hr at 25 °C	Equivalent to approx. 270 x g Centrifugation
3-10	3-10	3-10	3-10	3-10	3-10
50% suspension in 30% ethanol	50% suspension in 30% ethanol	Clear 96-well-flat bottom, polystyrene strip plate	Clear 96- and 384-well flat bottom, polystyrene plates	Clear 96-well flat bottom, polystyrene plates	Pre-packed dry matrix in 96-well filter plate
30% ethanol	30% ethanol	N/A	chlorhexidine	N/A	N/A
2-8 °C	2-8 °C	2-8 °C	2-8 °C	2-8 °C	2-8 °C
0-10 mM	0-10 mM	0-5 mM	0-1 mM	0-1 mM	0-5 mM
250 mM	250 mM	N/A	200 mM	200 mM	250 mM