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ProductInformation

HIS-Select™ Magnetic Agarose Beads

Product Number **H 1786**
Storage Temperature: 2 to 8 °C

TECHNICAL BULLETIN

Product Description

HIS-Select™ Magnetic Agarose Beads consist of paramagnetic, immobilized metal-ion affinity chromatography (IMAC) resin, designed for use in automated and small-scale affinity capture (molecular pull-down) purifications. The HIS-Select Magnetic Agarose Beads contain a proprietary, patent pending, quadridentate chelate, which is bound with nickel and covalently attached through a non-charged, hydrophilic linker to magnetic 6% beaded agarose.

HIS-Select Magnetic Agarose Beads are designed to capture proteins with histidine tags while exhibiting low non-specific binding of other proteins. The selectivity of the magnetic beads can be modulated with a low concentration (10 mM) of the histidine analog, imidazole, during capture and washing steps. Recombinant proteins with histidine tags can be captured under native or denaturing conditions.

The HIS-Select Magnetic Agarose Beads bind His-tagged recombinant proteins to allow their purification from cell lysates and other biochemical solutions in a similar manner as the standard HIS-Select HC Nickel Affinity Gel (Product No. P 6611). The His-tagged proteins, bound to the affinity resin, are separated with the use of a magnet. The magnetic properties allow for very rapid processing. These properties also aid in manipulations, such as repetitive washings, and recovery of the protein bound beads. This leads to faster recovery, experimental reproducibility, and more accurate quantitation of the proteins of interest.

The capacity of these magnetic beads is typically greater than 15 mg/ml of packed gel as determined with an approximately 30 kDa His-tagged protein. The matrix for these magnetic beads is 6% beaded agarose, with a diameter of 20-75 µm, average diameter 50 µm. Paramagnetic iron is impregnated within the beads.

Reagents

HIS-Select Magnetic Agarose Beads are supplied as a 50% slurry suspension in 30% ethanol (an antimicrobial preservative).

Equipment Required But Not Provided

Magnetic Separators:

For Microcentrifuge Tubes	M 1167
For Tissue culture flasks	M 1292
For Centrifuge Tubes	M 1542

Precautions and Disclaimer

HIS-Select Magnetic Agarose Beads are for laboratory use only, not for drug, household or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Note: Buffers or reagents that chelate metal ions should **not** be used with this product because they may strip the nickel ions from the beads. Strong reducing agents should also be avoided, because they may chemically reduce the bound nickel and thus eliminate the binding of histidine containing proteins. See the Reagent Compatibility Chart for more information.

Preparation Instructions
HIS-Select Magnetic Beads are stored in 30% ethanol. Thoroughly resuspend the affinity gel with gentle inversion and remove an aliquot for use. Take only the amount of affinity gel that is necessary for the purification to be done.

Prepare the following buffers for use in purification procedures for recombinant proteins with histidine containing tags. For native conditions, the equilibration buffer and wash buffer are the same.

1. Equilibration and Wash Buffer: 50 mM sodium phosphate, pH 8.0, 0.3 M sodium chloride, 10 mM imidazole

2. Elution Buffer: 50 mM sodium phosphate, pH 8.0, 0.3 M sodium chloride, 250 mM imidazole

Storage/Stability
Store the HIS-Select Magnetic Agarose Beads as supplied at 2 to 8 °C. The unopened product is stable for at least two years.

Regenerated resin should always be stored in a solution containing 30 % ethanol and kept at 2 to 8 °C for maximum stability.

Procedure
Affinity capture of His-tagged proteins
Note: It is recommended that the entire technical bulletin be read before use, especially the Reagent Compatibility Chart at the end of this bulletin.

There are many different procedures for performing small-scale affinity capture experiments. The procedure below is written for a single sample and is appropriate for most cell lines. The exact method used should be determined and optimized by the investigator, depending on the source of a particular sample (e.g., bacteria, fungi, plant cells, or tissue type). See Reference 1 for additional information and procedures.

For most affinity capture reactions, Sigma recommends using of 20 µl of the gel suspension/well (10 µl packed gel) for automatic 96-well purification procedures. For pull-down purification, 100 µl of the gel suspension/reaction (~50 µl of packed gel) is recommended. The amount of resin can be varied, depending on the amount of target protein in the sample and the type of magnetic separator utilized.

Volume of Bead Suspension (µl)	Binding Capacity
10	~75 µg
20	~150 µg
50	~375 µg
100	~750 µg
200	~1.5 mg

Extract Preparation
The recombinant protein with a histidine containing tag may be extracted from a crude cell extract or a partially purified protein fraction prepared by standard techniques. Users should empirically determine the protein sample preparation steps best suited to their samples, because the conditions may vary depending on the nature of the recombinant protein and the host organism.

CelLytic B (Product No. B 3553) or CelLytic B-II (Product No. B 3678) supplemented with 1 to 20 mM imidazole (Product No. I 0125) is recommended for use in *E. coli* cell lysis. Prior to application to the affinity gel, the recombinant protein sample must be clarified by centrifugation or filtration. For optimal results, the pH of the sample buffer must be between pH 7.0 and 8.0. The sample buffer should be supplemented with 1 to 20 mM imidazole and 0.15 to 0.5 M sodium chloride to reduce non-specific protein binding. Consult the reagent compatibility chart for the use of other reagents.

Controls: For a negative control to monitor non-specific binding, use a comparable volume of lysate from cells not expressing the His-tagged protein. Also, high levels of imidazole (150 mM to 250 mM) can be added to the affinity capture reaction as a competitor to test the binding specificity of the target protein.

1. Carefully mix HIS-Select Magnetic Agarose Beads until uniformly suspended. Immediately add required amount of the suspension to the lysate containing the his-tagged protein. To dispense beads, use a wide orifice pipette tip. Gently mix the material on a plate shaker or an orbital shaker (~175 rpm) for 30 minutes. Avoid magnetic stir bars.

2. Place the plate or tube in the magnetic separator for 10 seconds. Remove the supernatant and save for SDS-PAGE analysis.

3. Add 10 volumes of wash buffer to the affinity gel.

4. Mix the affinity gel suspension on a plate shaker or orbital shaker (~175 rpm) for 1 minute. Apply the magnetic separator and remove the supernatant.

5. Repeat steps 3 and 4 to wash the affinity gel again.

6. If desired, the affinity gel can be washed further until the A_{280} of the eluate no longer decreases. Discard the washes.

7. Add 5 gel volumes of elution buffer. Mix the affinity gel on a plate shaker or an orbital shaker (~175 rpm) for 15 minutes.

8. Apply magnetic separator, remove the supernatant and save. The histidine-containing protein will be in this fraction.

Denaturing Conditions

HIS-Select Magnetic Agarose Beads can be used to purify proteins under denaturing conditions. If denaturing conditions must be used, the protein must first be solubilized with 6 M guanidine hydrochloride (Product No. G 3272) or 8 M urea (Product No. 1250). Make sure the pH of the denatured cell extract is between pH 7.0 and 8.0 before applying it to the affinity gel. The same purification procedures employed above can be used with denaturing buffers. Note: Any buffers that contain urea must be prepared daily.

An example of a urea denaturing system is described below:

Equilibration Buffer:
0.1 M sodium phosphate pH 8.0, 8 M urea

Wash Buffer:
0.1 M sodium phosphate, pH 6.3, 8 M urea

Elution Buffer:
0.1 M sodium phosphate, pH 4.5 to 6.0, 8 M urea

The pH of the elution buffer may have to be varied because some His-tagged recombinant proteins will not elute in the pH 5.0 to 6.0 range. If the tagged recombinant proteins will not elute in this range, try a pH as low as 4.5.

References

1. Ausubel F. M., et al. *Current Protocols in Molecular Biology*, pp. 10.11.8-10.11.21 (John Wiley and Sons Inc., NY, 1998).
2. Porath, J., et al., Metal chelate affinity chromatography, a new approach to protein fractionation. Nature, **258,** 598-599 (1975).
3. Porath, J., and Olin, B., Immobilized metal ion affinity adsorption and immobilized metal ion affinity chromatography of biomaterials. Serum protein affinities for gel-immobilized iron and nickel ions. Biochemistry, **22**, 1621-1630 (1983).
4. Sulkowski, E., Purification of proteins by IMAC. Trends Biotechnol., **3**, 1-12 (1985).
5. Anderson, L., et al., Facile resolution of α-fetoproteins and serum albumin by immobilized metal affinity chromatography. Cancer Res., **47**, 3624-3626 (1987).
6. Hemdan, E. S., et al., Surface topography of histidine residues: a facile probe by immobilized metal ion affinity chromatography. Proc. Natl. Acad. Sci. USA, **86**, 1811-1815 (1989).
7. Sulkowski, E., Immobilized metal ion affinity chromatography of proteins. In: *Protein Purification: Micro to Macro*, R. Burgess (Ed.), pp. 149-162 (Alan R. Liss, Inc., New York, 1989).

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Reagent Compatibility Chart

Reagent	Effect	Comments
Imidazole	Binds to the nickel affinity gel and competes with histidine containing proteins	For weakly binding histidine-containing proteins and tags (e.g., the HAT tag), no more than 10 mM is suggested in the lysis and wash buffers to prevent non-specific binding of proteins. For strongly binding histidine-containing proteins and tags, no more than 30 mM is suggested in the lysis and wash buffers to prevent non-specific binding of proteins. The imidazole concentration may be reduced or eliminated; however, this may lead to increased binding of naturally occurring proteins containing histidine rich domains. High concentrations of imidazole (100 mM to 250 mM) may be used to elute captured target proteins from the beads.
Histidine	Binds to the nickel affinity gel and competes with the histidine containing proteins	Can be used in place of imidazole in the lysis, wash, and elution buffers. No more than 250 mM is suggested for the elution buffers.
Glycine	Binds weakly to affinity gel and competes weakly with histidine containing proteins	Not recommended; Sigma recommends the use of histidine or imidazole instead.
Chelating agents (e.g. EDTA, EGTA)	Removes nickel ions from the affinity gel	Not recommended as a buffer component, because of its ability to remove nickel ions. Can be used to strip nickel ions from the affinity beads to reveal non-specific protein binding to the affinity bead.
Guanidine HCl	Solubilize proteins	Use 6 M guanidine HCl for purification under denaturing conditions.
Urea	Solubilize proteins	Use 8 M urea for purification under denaturing conditions.
Sodium phosphate	Used in wash, and elution buffers to help prevent non-specific binding and buffer the solution	Recommended buffer at 50 to 100 mM for purification with the affinity gel. The pH of any buffer should be between 7 and 8 with the higher capacity target protein binding at the higher pH.
Sodium chloride	Prevents ionic interactions	Used in wash and elution buffers to help prevent non-specific binding of proteins to the affinity gel. Recommended levels are 0.15 to 0.5 M, but up to 2 M can be used.
β-Mercapto-ethanol	A reducing agent used to prevent disulfide bonds formation.	Add up to 20 mM in the lysis buffer to break disulfide bonds. Higher levels may reduce the nickel ions.
DTE, DTT	Reduces nickel ions	Not recommended.
Ethanol	Antimicrobial Also eliminates hydrophobic bonds between proteins	The binding, washing, eluting, and storage buffers may contain up to 30 % ethanol.
Glycerol	Can help stabilize proteins	The binding, washing, eluting, and storage buffers may contain up to 50 % glycerol.
Nonionic detergents (Triton®, TWEEN®, Igepal® CA-630)	Helps prevent non-specific binding of proteins to the affinity gel	Up to 2 % may be used.

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