Technical Bulletin

HIS-Select® Nickel Magnetic Agarose Beads

H9914

Product Description

HIS-Select® Nickel Magnetic Agarose Beads consist of a 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® Nickel Magnetic Agarose Beads contain a proprietary quadridentate chelate, which is bound with nickel and covalently attached through a non-charged, hydrophilic linker to magnetic 4% beaded agarose.

HIS-Select® Nickel 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 imidazole, a histidine analog, during capture and washing steps. Recombinant proteins with His-tags can be captured under native or denaturing conditions.

The HIS-Select® Nickel 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® Nickel Affinity Gel (Cat. No. P6611). The His-tagged proteins, after binding to the HIS-Select® Nickel Magnetic Agarose affinity resin, are separated with the use of a magnet. The magnetic properties allow for rapid processing. These properties also aid in downstream steps, such as repetitive washings and recovery of the protein-bound beads. The matrix for these magnetic beads is 4% beaded agarose, with a diameter of 20-75 µm (average diameter, 50 µm). Paramagnetic iron is impregnated within the beads.

Several publications[5-11] and dissertations[12-14] have cited use of this H9914 product in their research protocols.

Reagent

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

The capacity of these magnetic beads is typically > 10 mg/mL of packed gel, as determined with an ~30 kDa His-tagged protein.

Reagents and Equipment Required but Not Provided

Suggested Cat. Nos. are provided as appropriate.

- Magnetic Separators for:
  - Microcentrifuge tubes (such as Cat. No. M1167)
  - Tissue culture flasks
  - Centrifuge tubes
- Magnet for 96-well tissue culture plates (Cat. No. SHM05)
- Magnetic plate for standard-sized well plates, T-25 through T-75 tissue culture flasks, and up to 5 cm dishes (Cat. No. SHM04)

Storage/Stability

Store the HIS-Select® Nickel Magnetic Agarose Beads as supplied at 2-8 °C.

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

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.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

HIS-Select® Nickel 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 to do the purification.

Prepare the following buffers for use in purification procedures for His-tagged recombinant proteins. For native conditions, the Equilibration Buffer and Wash Buffer are the same.

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

Elution Buffer: 50 mM sodium phosphate, pH 8.0, with 0.3 M sodium chloride and 250 mM imidazole

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 following procedure 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 (bacteria, fungi, plant cells, mammalian tissue, etc.). See Reference 1 for additional information and procedures.[1]

- For most affinity capture reactions, use of 20 µL of the gel suspension/well (10 µL packed gel) for automated 96-well purification procedures is recommended.
- 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.

Packed Gel Volume	Binding Capacity
10 µL	~100 µg
20 µL	~200 µg
50 µL	~500 µg
100 µL	~1.0 mg
200 µL	~2.0 mg

Extract Preparation

The His-tagged recombinant protein 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 optimal for their samples, because the conditions may vary depending on the nature of the recombinant protein and the host organism.

CelLytic™ B (Cat. No. B7435) supplemented with 1-20 mM imidazole (such as Cat. No. I5513) 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 7.0-8.0. The sample buffer should be supplemented with 1-20 mM imidazole and 0.15-0.5 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 that do not express the His-tagged protein. Also, high levels of imidazole (150-250 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® Nickel Magnetic Agarose Beads until uniformly suspended. Immediately add required volume of the suspension to the lysate that contains 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. 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 the magnetic separator. Remove and save the supernatant. The His-tagged protein will be in the supernatant.

Denaturing Conditions

HIS-Select® Nickel 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 (such as Cat. No. G3272) or 8 M urea (such as Cat. No. U5378). Make sure the pH of the denatured cell extract is between pH 7.0-8.0 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.

The following is an example of a urea denaturing system:

- Equilibration Buffer: 0.1 M sodium phosphate, pH 8.0, with 8 M urea
- Wash Buffer: 0.1 M sodium phosphate, pH 6.3, with 8 M urea
- Elution Buffer: 0.1 M sodium phosphate, pH 4.5-6.0, with 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 5.0-6.0 range. If the His-tagged recombinant proteins will not elute in this range, try a pH as low as 4.5.

References

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Notice

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Reagent Compatibility Table

Reagent	Effect	Comments
Imidazole	Binds to the nickel affinity gel and competes with histidine-containing proteins	• For weakly binding histidine-containing proteins and tags (such as 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 that contain histidine-rich domains. • High concentrations of imidazole (100-250 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**. The use of histidine or imidazole is recommended instead.
Chelating agents (such as EDTA or EGTA)	Removes nickel ions from the affinity gel	• **Not recommended** as buffer components, because of their 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 50-100 mM for purification with the affinity gel. • The pH of any buffer should be between 7-8, 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 0.15-0.5 M, but up to 2 M can be used.
2-Mercaptoethanol	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.