Technical Bulletin

EZview™ Red HIS-Select® HC Nickel Affinity Gel

E3528

Product Description

EZview™ Red HIS-Select® HC Nickel Affinity Gel is a highly visible, red-colored, immobilized metal-ion affinity chromatography (IMAC) resin, designed for use in small-scale affinity capture (molecular pulldown) experiments. The HIS-Select® HC Nickel Affinity Gel is a proprietary quadridentate chelate, which is bound with nickel and covalently attached to 6% beaded agarose.

This affinity gel is designed to bind specifically to histidine-containing sequences in proteins. HIS-Select® HC Nickel Affinity Gel is selective for recombinant proteins with histidine tags (His-tags) and exhibits low non-specific binding of other proteins. The selectivity of the affinity resin can be modulated by the histidine analog imidazole during the capture and washing steps. Recombinant proteins with His-tags can be captured under native or denaturing conditions.

The EZview™ Red HIS-Select® HC Nickel Affinity Gel binds histidine (His)-containing proteins and His-tagged recombinant proteins, to allow their purification from cell lysates and other biochemical solutions in the same manner as the standard HIS-Select® HC Nickel Affinity Gel (Cat. No. P6611). The His-tagged proteins, bound to the affinity resin, are separated by centrifugation. The red color enhances visibility of the gel to facilitate downstream steps, such as repetitive washings, and recovery of the protein-bound beads. Several publications[8] and dissertations[9-10] have cited use of this E3528 product in their research protocols.

Reagent

EZview™ Red HIS-Select® HC Nickel Affinity Gel is supplied as a ~50% slurry suspension in phosphate buffered saline (PBS), pH 7.2, 50% (v/v) glycerol, and 0.0015% (v/v) (15 ppm) Kathon® CG/ICP II (an antimicrobial preservative).

Binding capacity: >15 mg/mL of packed gel

Equipment Required but Not Provided

Suggested Cat. Nos. are provided as appropriate.

- Cells to be used for preparation of lysate
- Appropriate lysis buffer, such as:
  - CelLytic™ M (Cat. No. C2978)
  - CelLytic™ MT (Cat. No. C3228)
  - CelLytic™ B (B7435, B7310, or C8740)
  - CelLytic™ P (C2360)
  - CelLytic™ Y (C4482)
  - RIPA Buffer (Cat. No. R0278)
- Vortex mixer
- Protease Inhibitor Cocktail suitable for use in purification of His-tagged proteins, such as:
  - Cat. No. P8849 (DMSO solution)
  - Cat. No. S8830 (tablets, EDTA-free)
  - Cat. No. PIC0004 (ReadyShield®, non-freezing solution)
- Pipette tips (200 µL)
- Pipette tips, wide orifice (200 µL)
- Pipette tips (1,000 µL)
- Pipette (200 µL)
- Pipette (1,000 µL)
- Microcentrifuge tubes (such as Cat. No. T9661)
- 2× Laemmli Sample Buffer (Cat. No. S3401)

Storage/Stability

It is recommended to store the EZview™ Red HIS-Select® HC Nickel Affinity Gel as supplied at -20 °C. The unopened product is stable for at least one year. The resin should always be stored in a solution containing 50% glycerol and should be kept at -20 °C for maximum stability.

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.

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 on Page 4 of this bulletin.

There are many different procedures to perform small-scale affinity capture experiments. This procedure is written for a single sample and is appropriate for most mammalian tissue culture cell lines. The investigator should determine and optimize the most appropriate method, depending on the source of a particular sample (such as bacteria, fungi, plant cells, or tissue type). See Reference 1 for additional information and procedures.[1]

For multiple affinity capture reactions, calculate the volume of reagents needed according to the number of samples to be processed. For easy performance of affinity capture reactions, it is recommended to use 40 µL of the gel suspension per reaction (~20 µL of packed gel). The amount of resin can be varied, depending on the amount of target protein in the sample.

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 EZview™ Red HIS-Select® HC Nickel Affinity Gel beads until completely and uniformly suspended. Immediately aliquot 40 µL of the 50% slurry into a clean 1.5 mL microcentrifuge tube on ice. To dispense beads, use a wide orifice pipette tip.
2. Wash/Equilibrate beads:
   - Add 750 µL of ice-cold lysis buffer to the tube containing the beads. Vortex. Centrifuge in a microcentrifuge for 30 seconds at 8,200 × *g* (10,000 rpm in an Eppendorf® 5415C microcentrifuge).
   - Carefully remove the supernatant with a micropipette, or carefully aspirate the supernatant.
   - Set the tube with the bead pellet on ice.

   Note: Because of the enhanced visibility of the affinity resin beads, it is easy to see if beads have been accidentally removed during any wash steps. If this happens, simply return the wash supernatant to the tube, and repeat the centrifugation step to pellet the resin again.
3. Repeat Wash Step #2. After removing the supernatant, set the washed bead pellet on ice.

   Notes:
   - For numerous affinity capture samples, the resin needed for all samples can be washed together.
   - Each wash should be performed with lysis buffer at a volume of at least 15 times the total packed gel volume.
   - The washed resin can then be aliquoted for the desired number of samples.
4. Prepare cell lysate using ice cold lysis buffer.
   - For most mammalian cells, 0.5-5 × 10^7 cells can be easily lysed in 1 mL of lysis buffer.
   - To minimize non-specific binding of lysate proteins to the gel matrix, the ionic strength of the buffer may be increased by increasing the NaCl concentration up to 500 mM.
   - Adding low levels of imidazole will reduce the binding of some endogenous His-containing proteins:
     - To purify weakly binding His-tagged (such as the HAT® tag) or histidine-containing proteins, add imidazole to a final concentration of 3-5 mM.
     - For strongly binding histidine tags or histidine-containing proteins, add imidazole to 20-30 mM final concentration.
   - Include an appropriate protease/phosphatase inhibitor cocktail to the lysis buffer if desired.
5. Transfer 1 mL of the lysate to an empty 1.5 mL microcentrifuge tube. Clarify the lysate of denatured protein and cell debris by centrifugation, at 2-8 °C, for 10 minutes at 8,200 × *g*. Add 1 mL of the cell lysate supernatant to the washed resin.
6. Agitate or shake all samples and controls gently (a rotating wheel apparatus is recommended) for 1 hour at 2-8 °C.
7. Centrifuge in a microcentrifuge for 30 seconds at 8,200 × *g*. Set the tube on ice. Remove the supernatant carefully. Set the tube with the bead pellet on ice.

8. Wash the bead pellet:
   - Add 500 µL of lysis buffer.
   - Vortex briefly. Incubate with thorough, gentle mixing at 2-8 °C for 5 minutes.
   - Centrifuge in a microcentrifuge for 30 seconds at 8,200 × *g*.
   - Aspirate supernatant carefully or remove with a micropipette.
   - Store the tube with the bead pellet on ice.
9. Repeat the Step 8 wash two or three more times. After removing the final wash supernatant, the bound target protein is ready for elution.

Elution of bound proteins:

A. General Elution

- The bound proteins may be eluted directly with high levels (150-250 mM) of imidazole in the buffer of choice.
- Note: Some enzyme assays, such as kinase assays, can be performed directly in the tube with the bead sample by directly adding the assay reaction mixture and substrate to the tube. The bead pellet first should be equilibrated by washing with the assay buffer before adding assay components.

B. Elution for SDS-PAGE analysis

- To elute bound proteins from the bead pellet for SDS-PAGE analysis, add 25 µL of the lysis buffer to the tube, and vortex briefly. Then add 25 µL of 2× Laemmli sample buffer.
- Vortex briefly.
- Boil the samples for 5 minutes. Vortex. Centrifuge for 30 seconds at 8,200 × *g* in a microcentrifuge to pellet the EZview™ Red HIS-Select® HC Nickel Affinity Gel beads.
- If not to be used immediately, the eluted bead samples may be frozen until the SDS-PAGE is run.
- Run 10-20 µL of the supernatant on a denaturing SDS/PAGE gel.
- Perform subsequent analysis by staining, autoradiography, or immunoblotting, as desired.

Note: For analysis using non-reducing SDS/PAGE, use a sample buffer without reducing agents such as 2-mercaptoethanol or dithiothreitol.

References

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5. Anderson, L. *et al.*, *Cancer Res.*, **47(14)**, 3624-3626 (1987).
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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 5 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 (100-250 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 a buffer component, 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 equilibration, 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 equilibration, 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 a disulfide bond. Higher levels may reduce the nickel ions.
DTE or 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 20% ethanol.
Glycerol	Can help stabilize proteins	The binding, washing, eluting, and storage buffers may contain up to 50% glycerol.
Nonionic detergents (TRITON®, TWEEN®, or IGEPAL® CA-630)	Helps prevent non-specific binding of proteins to the affinity gel	Up to 2% may be used.

Troubleshooting Guide

Problem	Possible Cause	Solution
Poor recovery of target protein	Histidine-containing protein is not present in the sample.	• Make sure the sample is appropriate. Analyze for the presence of histidine-containing protein by immunoblot or dot blot analyses. • Prepare fresh lysates. Avoid using frozen lysates. • Use appropriate protease/phosphatase inhibitors in the sample. If necessary, increase the concentration of the inhibitors to prevent degradation of the histidine-containing protein.
	Washes are too stringent.	• Reduce the number of washes. • Avoid adding high concentrations of NaCl to the mixture. • Use solutions that contain less or no detergent.
	Incubation times are inadequate.	Increase the incubation times with the affinity resin (from several hours to overnight).
	Interfering substance is present in sample.	• Lysates that contain EDTA or other metal chelators will remove the nickel from the affinity gel and must be avoided. • Excessive detergent concentrations and other substances can interfere with protein interaction with the affinity gel.
	Detection system is inadequate.	If Western blotting detection is used: • Check primary and secondary antibodies using proper controls to confirm binding and reactivity. • Verify that the transfer was adequate by staining the membrane with Ponceau S. • Use fresh detection substrate or try a different detection system.
Background is too high.	Proteins bind non-specifically to the affinity beads or the microcentrifuge tubes.	• Vary the imidazole concentration to minimize non-specific protein binding, while maintaining target histidine-containing protein binding. • After suspending beads for the final wash, transfer entire sample to a clean microcentrifuge tube before centrifugation and elution.
	Washes are insufficient.	• Increase the number of washes. • Extend the duration of the washes, incubating each wash for at least 15 minutes. • Increase the salt and/or detergent concentrations in the wash solutions. • Centrifuge at lower speed to avoid non-specific trapping of lysate proteins during the initial centrifugation of the affinity resin protein complexes.

Related Products

- Protease inhibitor cocktails without chelators (such as Cat. Nos. P1860, P8849, PIC0004, S8830)
- Phosphatase inhibitor cocktails (Cat. Nos. P2850, P5726, P0044)
- Bicinchoninic Acid (BCA) Kit for Protein Determination (Cat. No. BCA1)
- QuantiPro™ BCA Assay Kit (Cat. No. QPBCA)
- EZBlue™ Gel Staining Reagent (Cat. No. G1041)
- EZview™ Red Protein A Affinity Gel (Cat. No. P6486)
- EZview™ Red Protein G Affinity Gel (Cat. No. E3403)
- EZview™ Red ANTI-FLAG® M2 Affinity Gel (Cat. No. F2426)