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Product Information

IMAC-Select Affinity Gel

Catalog Number **I1408**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
IMAC-Select Affinity Gel is an immobilized metal affinity chromatography (IMAC) product. While most commonly used for purifying proteins it can also be used for purifying peptides. The gel matrix consists of 6% beaded agarose derivatized with a proprietary quadridentate chelate. IMAC-Select Affinity Gel is durable and can capture proteins that have an affinity for various metal ions. Although some naturally occurring proteins have affinities for some metals, many are given a recombinant histidine tag so that the protein of interest will have an affinity for weak to borderline Lewis acids, such as nickel or cobalt. Exposed cysteine and tryptophan residues may also bind, but are not as commonly used as histidine. Other metal ions, such as iron or gallium, may be used to purify phosphoproteins. IMAC-Select Affinity Gel is supplied as a metal-free gel and allows researchers to add a chelating metal of choice. The gel can also be used to remove metal contamination from a protein or peptide solution. The resin will have a metal binding capacity of 10 to 30 μmole/ml of resin.

It is recommended that the entire technical bulletin be read before use, especially the Reagent Compatibility Chart.

Reagent
IMAC-Select Affinity Gel is supplied as a 50% suspension in 30% ethanol.

Reagents and Equipment Required but not Provided
(Product Numbers have been given where appropriate)

- Centrifuge and/or filtration device
- Centrifuge tubes or chromatography column
- Sodium phosphate, Product No. S0751
- Sodium chloride, Product No. S3014
- Imidazole, Product No. I0125, (optional)
- EDTA, Product No. ED4S
- Guanidine HCl, Product No. G3272
- CelLytic B Cell Lysis Reagents, Product Nos. B7435, B7310, or C8740 for bacterial lysis; or CelLytic M, Product No. C2978, for mammalian cell lysis
- Zinc sulfate, Product No. Z4750 or
- Copper sulfate, Product No. C1297

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Do not allow the affinity gel to remain in any buffer for extended periods of time (>24 hours) unless it contains an anti-microbial agent such as 30% ethanol.

Note: Buffers or reagents that chelate metal ions should **not** be used with this product since they may strip the metal ions from the gel matrix. Strong reducing agents should also be avoided. They may reduce the bound metal and eliminate the binding of desired proteins. See Reagent Compatibility Chart for more information.

Preparation Instructions
The IMAC-Select Affinity Gel is stored in 30% ethanol. The ethanol must be removed just prior to use. Resuspend the affinity gel with gentle inversion and remove an aliquot for use. Take only the amount of affinity gel necessary for the purification to be done. The affinity gel may then be poured into a chromatography column for protein purification using standard techniques or handled batch-wise for trial scale or large-scale preparations. The ethanol storage solution must be removed as it may cause precipitation of some buffer salts. In general, the affinity gel is first washed with 1 to 2 volumes of deionized water to remove ethanol. The gel is charged with 2 to 3 volumes of a solution of the desired metal ion at a concentration of 10 mg/ml, and then equilibrated with 3 to 5 volumes of equilibration buffer.

A typical equilibration buffer for recombinant histidine tagged proteins consists of 50 mM sodium phosphate, 0.15 to 0.5 M sodium chloride, pH 8.0. Imidazole can be added at 1 to 20 mM, if desired. For phosphoproteins and peptides use a pH between 2 and 3.

Several methods are available to elute the protein from the column. A typical elution buffer for recombinant histidine tagged proteins may be 50 mM sodium phosphate, 0.15 to 0.5 M sodium chloride, 250 mM imidazole, pH 8.0. A similar elution can be achieved by using a gradient of increasing concentrations of imidazole. If an imidazole gradient is used, it is recommended that the pH remain constant. Another elution method would be to use a decreasing pH gradient where the buffer would consist of 50 mM sodium phosphate, 0.15 to 0.5 M sodium chloride, pH less than 8.0. A final pH of 3 to 4 is usually recommended. A final elution method would use a metal chelating reagent, such as EDTA or EGTA, added to the equilibration buffer. However, it is important to note that this method results in low specificity and the metal ion will be in the eluted fractions. The elution for phosphoproteins and peptides is done using a buffer with a high pH (pH 8 to 9).

Note: Do not use a metal chelating reagent if concerned about metal ions in the purified protein solution. A metal chelating reagent will remove the metal ions from the gel matrix along with the proteins.

Storage/Stability
IMAC-Select Affinity Gel is stable for at least one year when stored properly. The IMAC-Select Affinity Gel should be cleaned after each use as described below and an anti-microbial agent such as 30% ethanol should be added to the storage buffer.

Procedure

I. Extract Preparation
The following procedure is suggested for recombinant histidine-tagged proteins.[1,5,6,7] Researchers must determine optimal protocol for their specific application.

A protein with an affinity for the metal ion being used may be extracted from a crude cell extract or from a partially purified protein fraction prepared by standard techniques. The protein sample preparation procedure should be determined empirically by the researcher because conditions may depend on the nature of the protein and host organism.

CelLytic B Cell Lysis Reagents are recommended for lysis of *E. coli* cells. Prior to application to the affinity gel, clarify the protein solution by centrifugation or filtration.

For optimal purification of recombinant histidine tagged proteins, the sample buffer must be between pH 7.0 and 8.0. The equilibration and sample buffers can 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 other reagents that can be used.

II. Trial Scale Purification (mini-prep)
A trial scale experiment (less than 1 mg of target protein) should be performed before attempting a large-scale purification to determine if the standard operating conditions will work for the protein of interest.
All steps may be performed at room temperature or at 2 to 8 °C.

1. Add 25 to 50 μl of IMAC-Select Affinity Gel suspension to a microcentrifuge tube and centrifuge for 30 seconds at 5,000 x *g*.

2. Carefully remove the supernatant and discard.

3. Add 2 volumes of 10 mg/ml metal solution and mix well. Commonly used metals are copper and zinc, provided as sulfate salts.

4. Centrifuge for 30 seconds at 5,000 x *g*. Remove and discard the supernatant.

5. Wash the affinity gel by adding 2 volumes of deionized water and mixing well.

6. Centrifuge for 30 seconds at 5,000 x *g*. Remove and discard the supernatant.

7. Add 200 μl of equilibration buffer and mix well.

8. Centrifuge for 30 seconds at 5,000 x *g*. Remove and discard the supernatant.

9. Add 100 μl of clarified protein solution and gently mix for 1 minute. Centrifuge the mixture as above and save the supernatant.

10. Wash the affinity gel twice with at least 500 μl of wash buffer. Gently mix the affinity gel for 10 seconds, then centrifuge for 30 seconds at 5,000 x *g*. Save the wash buffer for analysis.

11. The target protein is eluted with 50 µl of elution buffer. Add the buffer to the affinity gel and mix well.

12. Centrifuge for 30 seconds at 5,000 x *g*.

13. Repeat steps 11 and 12 to recover more of the protein. Most of the protein will be eluted in the first 50 µl fraction, but some residual protein may be eluted in the second cycle. Save the two fractions as either one pool or as separate fractions.

14. Analyze all of the fractions by SDS-PAGE to determine if the target protein was bound to the affinity gel and eluted. If the target protein did not bind and elute from the affinity gel, refer to the Troubleshooting Guide. It may be necessary to repeat the trial under denaturing conditions.

III. Large Scale Purification
All steps may be performed at room temperature or at 2 to 8 °C.

Native Conditions: Column Chromatography

1. Transfer the appropriate amount of IMAC-Select Affinity Gel to a chromatography column. Wash the affinity gel with 2 volumes of deionized water and then add 2 volumes of 10 mg/ml metal solution. Commonly used metals are copper and zinc, provided as sulfate salts.

2. Wash column with 2 volumes of deionized water.

3. Equilibrate with 3 volumes of equilibration buffer. The surface of the equilibration buffer should not go below the top of the gel bed. Do not allow the affinity gel to remain in equilibration buffer for extended periods of time (>24 hours) without the addition of anti-microbial agents.

4. The amount of affinity gel required depends upon the amount of target proteins in the extract. The capacity of the affinity gel for the target protein should be determined for each protein to be purified.

5. Load the clarified crude extract onto the column at a flow rate of 2 to 10 column volumes/hour. It is recommended that the cell extract be loaded as soon as it is prepared and that the loading time should not exceed 6 hours. If loading time will be excessive, the protein binding may be performed using the batch format (Section III-B, Steps 1-10). Place the batch-loaded affinity gel in a column and proceed with the wash and elution steps, 6 and 7.

6. After all of the extract is loaded, wash the column with wash buffer. The flow rate of the wash buffer should be about 10 to 20 column volumes/hour. The column should be extensively washed until the A_{280} of the material elution from the column is stable and near that of the wash buffer.

7. The target protein is eluted from the column using 3 to 10 column volumes of elution buffer. Collect fractions and assay for the target protein. The flow rate of the elution buffer should be 2 to 10 column volumes/hour.

B. Native Conditions: Batch Purification Method
Note: Batch adsorption must be performed in the absence of imidazole. See Reagent Compatibility Chart.

1. Add the appropriate amount of affinity gel suspension to a large centrifuge tube. Centrifuge the mixture at 5,000 x *g* for 5 minutes to pellet the affinity gel and then discard the supernatant. Alternatively, remove the supernatant by filtration.

2. Resuspend the affinity gel in 2 gel volumes of water.

3. Collect the affinity gel by centrifugation or filtration.

4. Remove and discard the supernatant.

5. Add 2 volumes of 10 mg/ml metal solution and mix well. Commonly used metals are copper and zinc, provided as sulfate salts.

6. Centrifuge for 30 seconds at 5,000 x *g*. Remove and discard the supernatant.

7. Wash the affinity gel with 2 volumes of deionized water.

8. Equilibrate the resin with 10 gel volumes of equilibration buffer

9. Centrifuge for 30 seconds at 5,000 x *g*. Remove and discard the supernatant.

10. Add the cell extract to the affinity gel. Gently mix the material on an orbital shaker (approx. 175 rpm) for 15 minutes. Do not use a stir plate; the stir bar will break the affinity gel beads.

11. Centrifuge the mixture at 5,000 x *g* for 5 minutes or filter. Remove and save the supernatant for SDS-PAGE analysis.

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

13. Mix the affinity gel suspension on an orbital shaker (approx. 175 rpm) for 4 minutes. Centrifuge the suspension at 5,000 x *g* for 5 minutes or filter.

14. Repeat steps 11 and 12 to wash the affinity gel again.

15. The affinity gel can be washed further until the A_{280} of the eluate no longer decreases. Discard the washes.

16. Add 2 gel volumes of elution buffer. Mix the affinity gel on an orbital shaker (approx. 175 rpm) for 10 minutes.

17. Centrifuge the mixture at 5,000 x *g* for 5 minutes or filter. Remove and save the supernatant or filtrate. The target protein will be in this fraction.

18. Repeat steps 15 and 16 to elute more protein. Save the eluted fractions as a single pool or separate fractions.

IV. Denaturing Conditions

IMAC-Select Affinity Gel can be used to purify proteins under denaturing conditions when using either nickel or cobalt as the metal of choice. If denaturing conditions must be used, the protein must first be solubilized with 6 M guanidine hydrochloride or 8 M urea. 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 **fresh daily.**

V. Cleaning IMAC-Select Affinity Gel for Reuse

The affinity gel should be cleaned after every run to ensure proper functioning on the next use. All steps may be performed at room temperature or at 2 to 8 °C.

A. General Cleaning

1. Wash the affinity gel with 2 column volumes of deionized water.

2. Clean the affinity gel with 5 column volumes of 6 M guanidine HCl, pH 7.5. The flow rate should be no more than 5 column volumes per hour.

3. Remove the guanidine HCl solution by washing with 2 to 3 column volumes of deionized water.

4. For immediate use, re-equilibrate the affinity gel with 2 to 3 column volumes of equilibration buffer. If the gel is to be stored, wash with 1 to 2 column volumes of 30 % ethanol and store at 2 to 8 °C.

B. Recharging IMAC-Select Affinity Gel

If the IMAC-Select Affinity Gel turns to a brown or gray color, the bound metal has been reduced. The reduced metal must be removed and the affinity gel recharged using the following procedure. **Note: EDTA, in general, will not remove iron or reduced cobalt from the affinity gel. Fresh gel must be used.**

1. Wash the affinity gel with 2 column volumes of deionized water.

2. Clean the affinity gel with 5 column volumes of 6 M guanidine HCl, pH 7.5. The flow rate should be no more than 5 column volumes per hour.

3. Remove the guanidine HCl solution by washing with 2 to 3 column volumes of deionized water.

4. Wash the affinity gel with 5 column volumes of 0.1 M EDTA, pH 7.0 to 8.0.

5. Wash the affinity gel with 2 column volumes of deionized water.

6. Recharge the gel with the metal of choice or wash with 1 to 2 column volumes of 30% ethanol and resuspend in 30% ethanol for storage at 2 to 8 °C.

7. To recharge, add 2 column volumes of 10 mg/ml metal solution. Wash the affinity gel with 2 column volumes of deionized water. For immediate use, re-equilibrate the affinity gel with 2 to 3 column volumes of equilibration buffer. If the gel is to be stored, wash with 1 to 2 column volumes of 30 % ethanol and store at 2 to 8 °C.

Note: The affinity gel can also be cleaned with 0.2 M acetic acid, 1 to 2% SDS, or ethanol. The ethanol concentration can be as high as 100%, but the ethanol concentration must be gradually increased or decreased in stages. Each stage should have an increase in ethanol concentration of no more than 25% (v/v) (i.e. 25, 50, 75, 100, 75, 50, 25, 0%) to prevent rapid volume changes of the affinity gel.

Results

The binding capacity is dependent on the nature and size of the target protein, the type of metal being used, and the conditions used for the purification. Modification of conditions may enhance the binding capacity as well as the purity of the final product. See the Troubleshooting Guide for more recommendations.

Before running SDS-PAGE on samples containing guanidine HCl, precipitate the protein using TCA. This procedure will also concentrate a protein sample. Add 100% TCA solution (Product No. T0699) to the protein sample to give a final concentration of 10% TCA. Incubate the sample on ice for 15 minutes and centrifuge the sample at full speed for 15 minutes. Carefully remove the supernatant with a pipet and resuspend the pellet in SDS-PAGE sample buffer.

Related Products

- HIS-Select Cobalt Affinity Gel (H8162)
- HIS-Select Nickel Affinity Gel (P6611)
- HIS-Select HF Nickel Affinity Gel (H0537)
- PHOS-Select Iron Affinity Gel (P9740)

Additional HIS-Select and PHOS-Select products may be found on Sigma's website.

References

1. Ueda, E. K. M. et al., Current and Prospective Applications of Metal Ion-Protein Binding. J. Chromatogr. A, **988**, 1-23 (2003).

2. 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).

3. Hochuli, E., et al., A., New Metal Chelate Adsorbent Selective For Proteins and Peptides Containing Neighbouring Histidine Residues. J. Chromatogr., **411**, 177-184 (1987).

4. Andersson, L., et al., Facile Resolution of Alpha-Fetoproteins and Serum Albumins by Immobilized Metal Affinity Chromatography. Cancer Res., **47**, 3624-3626 (1987).

5. Porath, J., et al., Metal Chelate Affinity Chromatography, A New Approach to Protein Fractionation. Nature, **258**, 598-599 (1975).

6. Sulkowski, E., Purification of Proteins by IMAC. Trends in Biotechnology, **3,** 1-7 (1985).

7. Hemdan, E.S., et al., Surface Topography of Histidine Residues: A Facile Probe By Immobilized Metal Ion Affinity Chrmatography. Proc. Natl. Acad. Sci. USA, **86**, 1811-1815 (March 1989).

8. Posewitz, M. C., and Tempst, P., Immobilized Gallium (III) Affinity Chromatography of Phosphopeptides. Anal. Chem., **71**, 2883-2892 (1999).

Commonly Used Metals for IMAC and Their Applications

Metal	Application	Products
Cobalt	Purification of HIS-tagged proteins	HIS-Select Cobalt Affinity Gel (H8162)
Nickel	Purification of HIS-tagged proteins	HIS-Select Nickel Affinity Gel (P6611)
Copper	Usually for purification of HIS-tagged proteins and histidine containing peptides	IMAC-Select Affinity Gel (I1408) and copper sulfate (C1297)
Zinc	Usually for purification of HIS-tagged proteins	IMAC-Select Affinity Gel (I1408) and zinc sulfate (Z4750)
Iron	Purification of Phosphopeptides	Phos-Select Iron Affinity Gel (P9740)
Gallium	Purification of Phosphopeptides	IMAC-Select Affinity Gel (I1408) and gallium(III) sulfate (463892)

Reagent Compatibility Chart

Reagent	Effect	Comments
Imidazole	Binds to the metal charged affinity gel and competes with the recombinant proteins with histidine tags	For column chromatography, no more than 10 mM is suggested in the extract, equilibration, and wash buffers to prevent non-specific binding of proteins. No more than 250 mM is suggested for the elution buffers. Many proteins will elute with imidazole levels as low as 100 to 200 mM. For batch methods the imidazole concentration may have to be reduced or eliminated.
Histidine	Binds to the metal charged affinity gel and competes with the histidine-tagged proteins	Can be used in place of imidazole in the extraction, equilibration, wash, and elution buffers. No more than 250 mM is suggested for the elution buffers.
Chelating agents, e.g. EDTA, EGTA	Strips metal ions from the affinity gel	Not recommended as buffer components, because they can remove metal ions. Used to strip the affinity gel before recharging with fresh metal ions.
Guanidine HCl	Solubilize proteins	Use 6 M guanidine HCl to denature proteins and to clean the affinity gel.
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 to 100 mM for purification with the affinity gel. The pH of any buffer should be between 7 and 8 with the higher capacity at the higher pH.
Sodium chloride	Prevents ionic interactions	Used in equilibration, wash, and elution buffers to help prevent binding of non-specific proteins to the affinity gel. Recommended levels are 0.15 to 0.5 M, but up to 2 M can be used.
2-Mercapto-ethanol	A reducing agent used to prevent disulfide bonds formation	Add up to 20 mM in the extract buffer to break disulfide bonds. Higher levels may reduce the cobalt ions.
Ethanol	Antimicrobial Also eliminates hydrophobic bonds between proteins	The binding, washing, eluting, and storage buffers may contain up to 30% ethanol. Note: Ethanol may cause precipitation of some buffer salts. Buffers should be prepared and checked for salt precipitation before use
Glycerol	Can help stabilize proteins	The binding, washing, eluting, and storage buffers may contain up to 50% glycerol.
DTE, DTT	Reduces metal ions	Not recommended.
Nonionic detergents (TRITON®, TWEEN®, Igepal® CA-630)	Helps prevent non-specific binding of proteins to the affinity gel	Up to 2% may be used.
Glycine	Binds weakly to affinity gel and competes weakly with histidine containing proteins	Not recommended; use histidine or imidazole instead.

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Troubleshooting Guide

Problem	Cause	Solution
Recombinant protein with histidine tag will not bind to affinity gel	Incorrect conditions for binding	Verify the pH and composition of sample and equilibration buffers. Make sure there are no chelating or reducing agents present in the extraction buffer. If batch mode, remove imidazole.
	Recombinant protein is not present.	Run a Western blot of the extract to verify that the recombinant protein is present.
	The histidine tag is buried within the protein structure	Run the affinity purification under denaturing conditions.
	Cells not extracted	Verify the cell extract contains target protein. Increase stringency of lysis/extraction.
Protein elutes in the wash buffer before the elution buffer is introduced	Wash stringency is too high	Lower the imidazole concentration and verify that the pH is between 7 and 8.
	The histidine tag is buried within the protein structure	Run the affinity purification under denaturing conditions or alter buffer composition and/or pH.
Protein precipitates during purification	Temperature is too low	Run the column at room temperature.
	Protein aggregates	Add stabilizing agents such as 5 to 10 % glycerol, 0.1 % TRITON X-100, or TWEEN 20. Increase the sodium chloride concentration up to 2 M. Add up to 20 mM reducing agent such as 2-mercaptoethanol. Add metals or cofactors to stabilize protein. Verify ethanol has been washed from gel.
Pressure problems with column	Extract contains insoluble material	The protein extract must be free of insoluble material before it is loaded into the column. Insoluble material may be removed by centrifugation or filtration through a 0.45 μm membrane.
Affinity gel changes color	Extract exposure	During purification many protein extracts tend to discolor an affinity gel during the loading step. The original color will return after the wash or elution step.
	Needs to be recharged	The affinity gel was used and cleaned numerous times, so it is time to recharge the affinity gel with metal of choice.
	Loses color during run and does not regain it by the end of the run	Do not use oxidizing or reducing agents in any of the buffers or extracts. Avoid metal chelators. Strip and recharge the affinity gel with metal ions.
Histidine-tagged recombinant protein with will not elute from the affinity gel	Elution conditions are too mild	Increase the amount of imidazole. Add urea or guanidine HCl to the elution buffer. For a denaturing purification with pH elution make sure the pH is low enough to elute the tagged recombinant protein; adjust elution buffer to pH 4.5. Perform batch purification so that high protein concentrations are avoided.
Non-specific proteins elute with the histidine-tagged recombinant protein	Binding and wash conditions are not stringent enough	Increase the amount of imidazole in the extract and wash buffers up to 20 mM.
	Target protein is being degraded by proteases	Add protease inhibitor cocktail (Product Code P8849).
	Material is linked by disulfide bonds	Add reducing agent such as 2-mercaptoethanol, up to 20 mM.

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