

Pierce™ Pull-Down PolyHis Protein:Protein Interaction Kit

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Number**Description**

21277

Pierce Pull-Down PolyHis Protein:Protein Interaction Kit, contains sufficient materials for conducting 25 pull-down assays using a polyhistidine-tagged fusion protein as the bait

Kit Contents:

HisPur™ Cobalt Resin, 750µL settled resin (1.5mL of 50% slurry in 20% ethanol); binding capacity > 10mg His-tagged protein per milliliter of settled resin

Pierce Lysis Buffer, 250mL

Imidazole Stock Solution, 4M, 5mL

BupH™ Tris Buffered Saline, 1 pack, 25mM Tris•HCl, 0.15M NaCl; pH 7.2 when reconstituted with 500mL of ultrapure water

Pierce Spin Columns Accessory Pack, 27 columns with pre-inserted frit and top and bottom caps

Collection Tubes and Caps Accessory Pack, 100 graduated 2mL tubes and plug caps

Storage: Upon receipt store Pierce Lysis Buffer at room temperature. Store remaining kit components at 4°C. Kit is shipped at ambient temperature.

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Introduction

Elucidating gene function involves determining the function of each gene's encoded protein product. In the cell, proteins participate in extensive networks of protein:protein interactions. These interactions take the form of dynamic "protein machines", which assemble and disassemble in concert with an ever-changing influx of intra-, inter- and extracellular cues.¹ A preliminary step in understanding protein structure and function is to determine which proteins interact with each other, thereby identifying the relevant biological pathways. These pathways are further dissected for structure and function by experimental procedures such as epitope mapping, X-ray crystallography and enzymology of functional protein complexes.

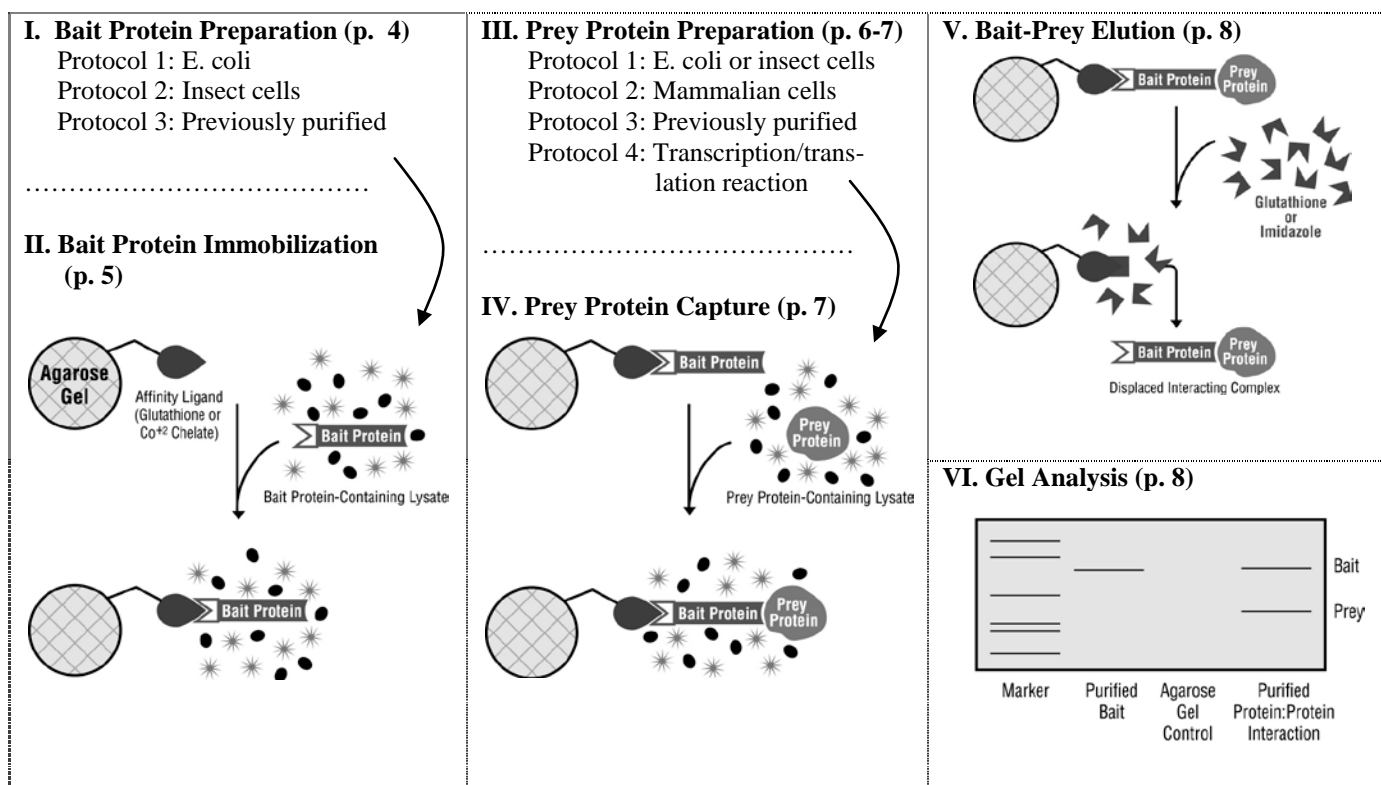
The polyhistidine pull-down technique has become an invaluable tool for the life scientist interested in protein chemistry. The basic pull-down assay is an *in vitro* technique that consists of a polyhistidine-tagged bait protein (the researcher's protein of interest) that can be used to identify putative binding partner(s) (the prey). The bait protein, purified from an appropriate expression system (e.g., *Escherichia coli* or baculovirus-infected insect cells), is immobilized to cobalt chelate affinity resin. The bait serves as the secondary affinity support for confirming a previously suspected protein partner or for identifying new protein partners to the bait. Prey protein can be obtained from multiple sources, including previously purified proteins, cell lysates or *in vitro* transcription/translation reactions. Protein:protein interactions can be visualized by SDS-PAGE and associated detection methods depending on the sensitivity requirements of the interacting proteins. These methods include coomassie, silver, and zinc staining; Western blotting; and [³⁵S] radioisotopic detection.² Experiments designed to identify new protein:protein interactions often entail protein band isolation from a polyacrylamide gel, tryptic digestion of the isolated protein and mass spectrometric identification of digested peptides.

Homemade pull-down methodologies for confirming or identifying protein:protein interactions are common in contemporary scientific literature.^{3,4,5} The homemade pull-down assay represents a collection of reagents from multiple commercial vendors that cannot be validated together as a functional assembly until the researcher incorporates them into a complete protocol. Troubleshooting this mix of reagents can be tedious and time-consuming. The Thermo Scientific™ Pierce™ Pull-Down PolyHis Protein:Protein Interaction Kit contains a complete, validated set of reagents specifically developed for performing pull-down assays. The kit format is highly flexible, allowing complete control in optimizing experimental conditions specific to the requirements of each unique protein:protein interaction.

Important Product Information

- It is often useful to establish experimental evidence that a protein of interest interacts with other proteins before attempting to perform a pull-down assay for binding partners. Several methods, including yeast two-hybrid, density gradient centrifugation and indirect immunofluorescence microscopy are valuable in predicting whether the protein of interest is involved in a complex with other proteins.
- Detailed protocols necessary to clone a gene with a polyhistidine tag or to express the polyhistidine-tagged gene product are available in numerous molecular biology and recombinant protein handbooks.²
- For best results, determine optimal conditions for overexpression of soluble polyhistidine-tagged fusion protein before attempting pull-down.
- Negative controls are absolutely necessary for generating biologically significant results. A non-treated gel control (minus bait, plus prey) helps identify and eliminate false positives caused by nonspecific binding of proteins to the HisPur Cobalt Resin. The immobilized bait control (plus bait, minus prey) helps identify and eliminate false positives caused by nonspecific binding of proteins to the polyhistidine tag of the bait protein. The immobilized bait control also serves as a positive control that verifies the cobalt affinity resin can successfully capture the polyhistidine-tagged bait protein.
- This kit incorporates the Thermo Scientific Pierce Spin Column format for handling small volumes of cobalt affinity resin. This format allows complete retention of resin during the pull-down assay and eliminates variability between experiments that results from resin loss (see Appendix C).⁶
- The buffer provided in the kit allows complete flexibility to determine optimal conditions for isolating interacting proteins. Characteristics of interacting proteins (See Appendix A) may require alteration of the binding and wash buffers by adding components such as protein cofactors or additional salts and detergents. However, different interacting pairs may require less stringent conditions and fewer binding reaction components. The working solution for washing and binding, a 1:1 mix of TBS to Thermo Scientific Pierce Lysis Buffer, is a physiologically neutral, Tris-buffered solution of 75mM NaCl with 1% of a proprietary nonionic detergent. This buffer provides a starting point from which specific buffer conditions for each unique interacting pair can be optimized.

Procedure Summary



Procedure for Pierce Pull-Down Polyhistidine Assay

Note: This kit and these instructions may be adapted to different formats depending on the source of bait and prey protein. The protocols are general guidelines and will require varying degrees of experimental analysis to determine optimal conditions for specific interaction pairs. Sufficient collection tubes are provided to perform the pull-down assay as described. More vigorous analysis of experimental conditions may require additional tubes and caps. Many tube styles will accommodate the spin columns. Rotor dimensions of each microcentrifuge will dictate the collection tube style that can be substituted. A 2mL collection tube (with a detached plug-style cap) allows sufficient clearance between the bottom opening of the Pierce Spin Column and the solution retained in the collection tube.

Additional Materials Required

- Pipettes and disposable tips that can accurately deliver 2µL to 1mL
- Vortex mixer
- 0.2µm, 500mL filter sterilization unit
- 1.5mL microcentrifuge tubes
- 2.0mL collection tubes
- Microcentrifuge capable of 12,000 × g
- Analytical balance capable of milligram quantitation

Buffer Preparation

Reconstitute contents of the Thermo Scientific BupH Tris Buffered Saline (TBS) pack with 500mL of ultrapure water. Filter sterilize the solution using a 0.2µm filter apparatus and store at 4°C. When stored properly, the reconstitution of one pack of BupH TBS is sufficient for 25 pull-down assays.

I. Bait Protein Preparation

Choose one of the following three protocols for polyhistidine-tagged bait protein preparation; then proceed to **Section II. Bait Protein Immobilization** on the next page.

Protocol 1: Bait from *E. coli* Expression Systems

1. Grow and transform *E. coli* according to standard protocols.
2. Transfer 5mL of IPTG-induced *E. coli* culture to a sterile centrifuge tube.
Note: This amount can be increased if the polyhistidine-tagged fusion protein exhibits low expression levels. Five milliliters of culture grown to $OD_{600} = 1.0$ (or greater) will suffice if the overexpressed polyhistidine-tagged fusion protein forms a distinguishable band when lysate is analyzed by SDS-PAGE and coomassie staining.
3. Centrifuge at $5000 \times g$ for 5 minutes and discard culture supernatant.
4. Resuspend pellet in 1mL of TBS per 5mL of original culture volume. Mix using a pipette or vortex mixer.
5. Transfer 1mL of cell suspension to a 1.5mL microcentrifuge tube.
6. Centrifuge at $5000 \times g$ for 5 minutes and discard supernatant.
7. Resuspend pellet in 200 μ L of ice-cold TBS per 5mL of original culture volume. Mix using a pipette or vortex mixer. Add protease inhibitors if desired. For optimal results, use protease inhibitor cocktail when preparing cell lysate.
8. Add 200 μ L of Pierce Lysis Buffer per 5mL of original culture volume. Immediately invert until thoroughly mixed.
Note: The Pierce Lysis Buffer will not solubilize inclusion bodies. For inclusion body solubilization, use Thermo Scientific Inclusion Body Solubilization Reagent (Product No. 78115).
9. Incubate on ice for ~30 minutes. Periodically invert tubes.
Note: Continue with **Section II. A. Equilibrate HisPur Cobalt Resin** while performing this incubation.
10. Centrifuge at $12,000 \times g$ for 5 minutes to clarify crude *E. coli* lysate.
11. Decant supernatant to a separate microcentrifuge tube and store on ice. Label this tube “bait lysate.”

Protocol 2: Bait from Insect Cell Expression Systems

1. Grow and infect insect cells according to standard protocols.
2. Transfer insect cell culture to a sterile centrifuge tube.
3. Centrifuge at $500 \times g$ for 5 minutes and discard culture supernatant.
4. Add 1mL of ice-cold TBS per 5mL of cultured insect cells. Invert tube several times to thoroughly suspend cells.
5. Transfer cell suspension to an appropriately sized centrifuge tube.
6. Centrifuge at $500 \times g$ for 5 minutes and discard wash supernatant.
7. Resuspend insect cell pellet with 2.5 volumes of ice-cold TBS per gram wet weight of insect cell pellet. Invert tube several times to thoroughly mix cells. For optimal results, use protease inhibitor cocktail when preparing cell lysate.
8. Add 2.5 volumes of Pierce Lysis Buffer per gram wet weight of insect cell pellet and immediately invert until thoroughly mixed.
9. Incubate on ice for ~30 minutes. Periodically invert tubes.
Note: Continue with **Section II. A. Equilibrate HisPur Cobalt Resin** while performing this incubation.
10. Centrifuge at $12,000 \times g$ for 5 minutes to clarify crude insect cell lysate.
11. Decant supernatant to a new microcentrifuge tube and store on ice. Label this tube “bait lysate.”

Protocol 3: Bait from Previously Purified Protein

1. Remove imidazole and/or EDTA from previously purified protein sample by dialysis against TBS.
2. Determine protein concentration of polyhistidine-tagged fusion protein sample using a protein assay method such as the Thermo Scientific BCA Protein Assay Kit (Product No. 23227) or Coomassie Plus Protein Assay Kit (Product No. 23236).

II. Bait Protein Immobilization

A. Equilibrate HisPur Cobalt Resin

1. Label a sufficient number of Pierce Spin Columns to include a sample, non-treated resin control and immobilized bait control for each experiment.
2. Prepare a 1:1 wash solution of TBS:Pierce Lysis Buffer and add the 4M Imidazole Stock Solution to a final concentration of 10mM imidazole. For each spin column, prepare ~8mL of wash solution and add 20 μ L of the 4M Imidazole Stock Solution.
3. Thoroughly resuspend the HisPur Cobalt Resin using a vortex mixer. Pipette 50 μ L of the slurry into each labeled spin column. For best results, use a cut or wide-bore pipette tip.

Note: HisPur Cobalt Resin is supplied as a 50% slurry. Settled resin volume per assay is 25 μ L.

4. Add 400 μ L of the wash solution to each spin column. Cap both ends of the column and invert several times to equilibrate the immobilized cobalt-chelate resin. Remove both caps and place spin column in a collection tube.
5. Centrifuge at 1250 \times g for 30 seconds to 1 minute. Replace bottom cap. Discard wash solution from collection tube and re-insert spin column.

Note: Wash solution can be retained for analysis, but this will require additional collection tubes (see note under Procedure for Pierce Pull-Down Polyhistidine Assay, p. 3).

6. Repeat wash Steps 4 and 5 for a total of 5 washes.

B. Immobilize Bait Protein

1. Apply bottom cap and remove top cap for each Pierce Spin Column.
2. Add prepared polyhistidine-tagged fusion protein (BAIT) to the Pierce Spin Column.
 - For cell lysates (Section I, Protocol 1 or 2), add at least 300 μ L of polyhistidine-tagged fusion protein lysate.

Note: Retain sufficient lysate to analyze the fusion protein expression by SDS-PAGE. If desired, determine lysate protein concentration using a BCA (Product No. 23227) or Coomassie Plus™ (Product No. 23236) Protein Assay Kit.
 - For previously purified polyhistidine-tagged fusion protein (Section I, Protocol 3), use a sufficient volume to ensure addition of 100-150 μ g of bait protein. (The Pierce Spin Column has a maximum capacity of ~800 μ L.)

Note: Twenty-five microliters of settled HisPur Cobalt Resin can bind > 250 μ g of polyhistidine-tagged protein. Larger fusion proteins may cause steric hindrance, blocking some immobilized cobalt sites from binding to the polyhistidine tag. Dilute protein solutions may have to be concentrated or applied to the column in multiple batches. For best results, dilute concentrated protein samples to at least 600 μ L with wash solution.

3. Replace top cap of each column.
4. Incubate at 4°C for at least 30 minutes with gentle rocking motion on a rotating platform. Maximal binding may require a longer incubation time, which should be determined for each new polyhistidine-tagged fusion protein.

Note: Continuing with **Section III. Prey Protein Preparation** while performing this incubation will shorten the total time for protocol completion.

5. Remove both caps from each column and place each into a collection tube.
6. Centrifuge at 1250 \times g for 30 seconds to 1 minute. Label this tube “bait flow-through” and place on ice.

7. Replace bottom cap on spin column.
8. Add 400 μ L of wash solution and replace top cap. Invert several times to mix thoroughly.
Note: Multiple washes with resin inversion are required to effectively eliminate nonspecific interactions. For additional information regarding interaction characteristics and wash requirements, see Appendix A, p. 9.
9. Remove both caps and place spin column in the collection tube.
10. Centrifuge at 1250 $\times g$ for 30 seconds to 1 minute. Label collection tube “wash,” discard the wash volume and reuse tube for all wash collections.
11. Repeat wash Steps 7-10 for a total of 5 washes.

III. Prey Protein Preparation

Choose one of the following four protocols for prey protein sample preparation; then, when bait protein has been prepared and immobilized (Sections I and II), proceed to **Section IV. Prey Protein Capture**.

Protocol 1: Prey Protein from *E. coli* or Baculovirus-Infected Insect Cell Lysate

For best results, determine optimal conditions for overexpression of soluble prey protein before attempting pull-down procedure. The confirmation of suspected protein:protein interactions may be validated using expression lysate proteins as the prey protein; however, identification of novel protein:protein interactions is not recommended.

1. Grow and transform *E. coli* or grow and infect insect cells according to standard protocols. Transfer cell culture to a sterile centrifuge tube. (Amount may be increased depending on expression level of protein.)
2. Centrifuge for 5 minutes and discard supernatant. (Centrifuge *E. coli* at 5000 $\times g$. Centrifuge insect cells at 500 $\times g$.)
3. Add 1mL of TBS per 5mL of cell culture. Mix using a pipette or vortex mixer.
4. Transfer cell suspension to a sterile centrifuge tube.
5. Centrifuge for 5 minutes and discard supernatant. (Centrifuge *E. coli* at 5000 $\times g$. Centrifuge insect cells at 500 $\times g$.)
6. Resuspend *E. coli* with 200 μ L of ice-cold TBS per 5mL of starting culture. Resuspend insect cells with 2.5mL of ice-cold TBS per gram wet weight of cells. For best results, use a protease inhibitor when preparing cell lysate.
7. Add 200 μ L of Pierce Lysis Buffer per 5mL of *E. coli* starting culture. Add 2.5mL of Pierce Lysis Buffer per gram wet weight of insect cells. Immediately invert until thoroughly mixed.
8. Incubate on ice for ~30 minutes. Periodically invert tubes.
9. Centrifuge at 12,000 $\times g$ for 5 minutes to clarify crude cell lysate.
10. Decant supernatant to a new tube and store on ice. Add the 4M Imidazole Stock Solution to a final concentration of 10mM imidazole. Immediately invert tube to thoroughly mix. Label this tube “prey lysate”

Protocol 2: Prey Protein from Mammalian Cell Lysate

Confirmation of suspected protein:protein interactions and identification of novel protein:protein interactions is possible using mammalian cell lysate as the source of prey protein.

1. Grow mammalian cells according to standard protocols.
2. Transfer cell culture to a sterile centrifuge tube.
Note: If cells are adherent, release them from the surface of the flask by trypsin digestion.
3. Centrifuge at 500 $\times g$ for 5 minutes and discard culture supernatant.
4. Add 1mL of TBS per 5mL of original cell culture volume. Mix using a pipette or a vortex mixer.
5. Transfer cell suspension to a sterile centrifuge tube.

6. Centrifuge at $500 \times g$ for 5 minutes and discard wash supernatant.
Resuspend mammalian cell pellet with 2.5mL of ice-cold TBS per gram wet weight of cells using a pipette or vortex mixer. For optimal results, use a protease inhibitor cocktail when preparing cell lysate.
7. Add 2.5mL of Pierce Lysis Buffer per gram wet weight of cells and immediately invert until thoroughly mixed.
8. Incubate on ice for ~30 minutes. Periodically invert tubes.
9. Centrifuge at $12,000 \times g$ for 5 minutes to clarify crude mammalian lysate.
10. Decant supernatant to a new tube and store on ice. Add the 4M Imidazole Stock Solution to a final concentration of 10mM imidazole. Immediately invert tube to thoroughly mix. Label this tube “mammalian prey lysate.”

Protocol 3: Prey from Previously Purified Protein

Confirmation of suspected protein:protein interactions may be validated using purified proteins as the bait and prey proteins. For *in vitro* translation, use the Thermo Scientific 1-Step Human Coupled IVT Kit (Product No. 88882).

1. Determine protein concentration of purified prey protein sample using the BCA Protein Assay Kit (Product No. 23227) or Coomassie Plus (Bradford) Protein Assay Kit (Product No. 23236).
2. Calculate the volume of purified prey protein sample that contains approximately 100-150µg of protein.
Note: This is a starting concentration and may have to be adjusted based on the molecular mass of the bait and prey protein as well as the nature of the interaction between the two proteins. The Pierce Spin Column has a maximum capacity of ~800µL. Previously purified protein may have to be concentrated or applied to the column in multiple batches for optimal binding. For concentrated protein samples, dilute sample up to 800µL with wash solution described in Section II A: Equilibrate HisPur Cobalt Resin, Step 2.

Protocol 4: Prey Protein from *In vitro* Transcription/Translation Reaction

Confirmation of suspected protein:protein interactions may be validated using *in vitro* transcribed/translated proteins as the prey protein; however, identification of novel protein:protein interactions is not recommended.

1. Perform *in vitro* transcription/translation reaction according to standard protocols.
Note: *In vitro* translated proteins are either [³⁵S]-labeled or biotinylated, therefore, autoradiography or Western blotting is required for detection. These reactions are typically 50µL.
2. Dilute 10µL of an *in vitro* transcription/translation reaction in up to 400µL of TBS/Pierce Lysis Buffer. Add the 4M Imidazole Stock Solution to a final concentration of 10mM imidazole. Immediately invert tube to thoroughly mix. This will serve as the prey protein sample.
Note: Efficiency of the *in vitro* transcription/translation reaction and strength of the protein:protein interaction being confirmed may require using more than 10µL of the reaction.

IV. Prey Protein Capture

1. Apply bottom cap and remove top cap on the Pierce Spin Column containing the immobilized polyhistidine-tagged bait protein.
2. Add up to 800µL of prepared prey protein sample.
Note: Retain enough sample to analyze prey protein via SDS-PAGE.
3. Replace top cap to the column.
4. Incubate at 4°C for at least 1 hour with gentle rocking motion on a rotating platform. Maximal binding may require a longer incubation time and should be determined for each new prey protein sample.
5. Remove both caps from each column and place column in a collection tube.
6. Centrifuge at $1250 \times g$ for 30 seconds to 1 minute. Label this tube “prey flow-through” and place on ice.
7. Replace bottom cap on spin column.

8. Add 400 μ L of wash solution and replace top cap. Invert several times to mix thoroughly.
Note: Multiple washes with resin inversion are required to effectively eliminate nonspecific interactions. For additional information regarding interaction characteristics and wash requirements, see Appendix A, p. 9.
9. Remove both caps and place spin column in the collection tube labeled “wash.”
10. Centrifuge at 1250 \times g for 30 seconds to 1 minute. Discard the wash volume.
11. Repeat wash Steps 7-10 for a total of 5 washes.
12. Proceed to **Section V. Bait-Prey Elution.**

V. Bait-Prey Elution

The following procedure is applicable to all methods of bait and prey preparations.

1. To prepare 1mL of 290mM Imidazole Elution Buffer, add 70 μ L of 4M Imidazole Stock Solution to 930 μ L of wash solution (which already contains 10mM imidazole). Prepare additional wash solution as needed.
2. Use a pipette or a vortex mixer to thoroughly mix Elution Buffer.
3. Apply bottom cap and remove top cap of spin column.
4. Add 250 μ L of the Elution Buffer to the spin column. Replace top cap to the column.
5. Incubate spin column for 5 minutes with gentle rocking on a rotating platform.
6. Remove both caps and place spin column in a collection tube.
7. Centrifuge at 1250 \times g for 30 seconds to 1 minute. Label this tube “Elution 1” and place on ice.
Note: Although multiple elutions may be performed for each assay, this is usually unnecessary because most interacting proteins will elute in the first elution. This kit contains sufficient 4M Imidazole Stock Solution for 3-4 elutions per assay so that a complete elution profile can be established for each protein assayed.
8. Prepare samples for SDS-PAGE.
Note: The high imidazole concentration in the Elution Buffer may cause lane widening during electrophoresis in Tris-glycine pre-cast gels. Sample dialysis with a Thermo Scientific™ Slide-A-Lyzer™ MINI Dialysis Unit (Product No. 69560) before SDS-PAGE analysis will eliminate this anomaly.

VI. Gel Analysis

1. Electrophorese samples, including non-treated HisPur Cobalt Resin and immobilized bait controls.
2. Choose a detection method based on sensitivity requirements.
3. Protein bands, which are not present in the control lanes, are candidate interacting proteins.

Troubleshooting

Problem	Possible Cause	Solutions
Expression levels of target protein in <i>E. coli</i> or baculovirus-infected insect cells are low	Expression conditions were not optimized	Optimize expression conditions
High background or many contaminating bands	Inadequate gel washing	Increase wash volume or ionic strength of wash solution
		Increase molar concentration of Imidazole in the wash solution by 10mM increments until a satisfactory purification is observed
No isolation of interacting protein	Weak or transient interaction	Wash conditions are too stringent; lower the number of washes and ionic strength of wash buffer
	Poor expression level of prey protein	Apply more protein sample
	Cofactor may be essential for “trapping” interaction	Experiment with addition of possible cofactors
Resin foams during wash	Top cap to column was still in place during centrifugation	Remove top cap to column

Appendix

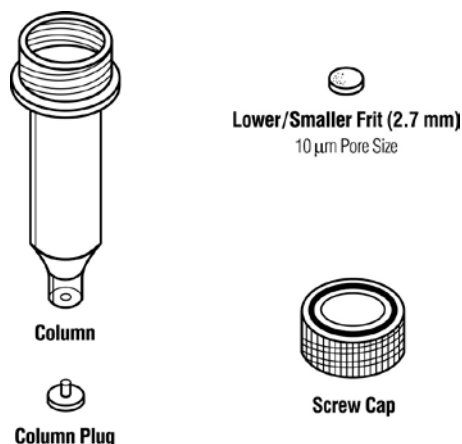
A. Considerations for Strong, Weak or Transient Protein:Protein Interactions

- **Strong:** Interactions that exhibit strong binding affinities (low dissociation constants) are the easiest to identify. These interactions may be washed extensively with high-ionic strength buffers to clear nonspecific proteins, eliminating false positive results.
- **Weak:** Interactions with weak binding affinities (high dissociation constants) require careful evaluation of binding and wash buffer conditions to allow the interaction to remain intact while removing nonspecific proteins. False positive results are more likely when identifying weak interactions. Eliminating false positive results requires careful design of appropriate control experiments.
- **Transient:** These interactions represent the most challenging to isolate and can be strong or weak in binding affinity, but are defined by their brief temporal interaction with other proteins. Transient interactions are common to enzymatic protein complexes that undergo dynamic rearrangements as the complex cycles through a particular biological process. A protein that transiently interacts with another protein or protein complex will often “dock” at a unique molecular interaction site. The recruited protein is held at this site only until it has performed its cognate enzymatic function after which it is allowed to recycle (disassemble from the complex) and await the next docking event. These transient interactions are frequently associated with NTP hydrolysis and, as such, the inclusion of NTP and non-hydrolyzable NTP analogs can be crucial to “trapping” a complex in a particular conformation conducive for the docking event under study. Other co-factors such as hormones and divalent cations can have similar effects on binding conformations depending on the system.

B. Endogenous Protein Expression

- Translation levels of different proteins, even ones in the same functional complex, can vary extensively when compared directly to each other, assayed during the cell cycle, and when calculated between different cell types. This variance can encompass a continuum from highly abundant to single molecule expression or no expression. Excessive translation of some proteins can potentially mask the interaction of other biologically significant interactions, while ultra-low expression can push the identification of biologically significant interactions below the threshold of current detection capabilities. These factors must be determined empirically for each protein.

C. Schematic of the Pierce Spin Column



Related Thermo Scientific Products

23227	BCA Protein Assay Kit
23236	Coomassie Plus™ (Bradford) Protein Assay Kit
24590	GelCode™ Blue Stain Reagent
24612	Pierce Silver Stain Kit
34080	SuperSignal™ West Pico Chemiluminescent Substrate
69705	Pierce Spin Columns Plus Accessories, 25 units
28376	BupH Tris Buffered Saline Packs, 40 packs

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