INSTRUCTIONS

MicroLink™ Protein Coupling Kit

PIERCE
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20475

1509.0

Number	Description
20475	**MicroLink™ Protein Coupling Kit,** contains sufficient reagents for 10 coupling reactions using 25-100 µg of antibody or antigen and 20 affinity purifications

Kit Contents:

AminoLink® Plus Coupling Gel Spin Columns, 10 each, columns contain 400 µl of 25% slurry

Coupling Buffer: BupH™ Phosphate Buffered Saline Pack, 1 pack, results in 0.1 M sodium phosphate, 0.15 M NaCl; pH 7.2 when reconstituted with 500 ml of ultrapure water

Quenching Buffer, 60 ml, 1 M Tris•HCl, 0.05% NaN_3, pH 7.4

Sodium Cyanoborohydride Solution (5 M), 0.5 ml, dissolved in 0.01 M NaOH

Wash Solution, 25 ml, contains 1 M NaCl, 0.05% NaN_3

Elution Buffer, 50 ml, pH 2.8, contains primary amine

Microcentrifuge Collection Tubes, 200 each

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

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Introduction

MicroLink™ Protein Coupling Kit allows immobilization of small amounts (25-100 µg) of purified antibody and other proteins directly onto beaded agarose gel to create a permanent affinity support. The AminoLink® Plus Coupling Gel included in this kit contains aldehyde functional groups that react with primary amines present on antibodies and other molecules. Reductive amination of the resulting Schiff bases forms a stable secondary amine linkage with minimal leakage. Crude sample is incubated with the immobilized protein to form an immune complex. The gel-bound complex is then washed to remove irrelevant material. Finally, the immune complex is dissociated. The purified product can be used for applications such as protein assays, ELISAs, binding studies and Western blotting.

MicroLink™ Protein Coupling Kit provides advantages compared to using typical affinity purification methods. Antibody immobilization allows faster and easier immunoprecipitations, enables reuse of the antibody, and results in purified antigen free from antibody contamination. This method features coupling of all primary amine-containing molecules and antibody species and subclasses, in contrast to being limited to antibody species that bind strongly to Protein A or Protein G.

Important Product Information

- The sample solution must not contain amines (e.g., Tris or glycine) as they will quench the reaction. Remove amines before coupling by dialysis using a Slide-A-Lyzer® Cassette (e.g., Product No. 66383) or a Slide-A-Lyzer® MINI Dialysis Unit (e.g., Product No. 69576).
- Gelatin or other carrier proteins in the sample solution will compete for coupling sites. Remove gelatin and carrier proteins by performing a Protein A or Protein G purification and subsequent dialysis using PBS. Alternatively, inquire with the antibody supplier if a carrier-/gelatin-free antibody is available.

Material Preparation

Coupling Buffer: Dissolve the dry-blend buffer with 500 ml of ultrapure water. For long-term storage of excess buffer, sterile-filter the solution or add sodium azide to a final concentration of 0.02% and store at 4°C.

Protein Sample: Dissolve protein or peptide to be immobilized with 200-300 µl of Coupling Buffer at 0.5-1 mg/ml. If the sample is not soluble in Coupling Buffer, dissolve it in a suitable buffer (see the Troubleshooting Section). For proteins already in solution, make a 1:1 dilution of the sample with Coupling Buffer or dialyze against the Coupling Buffer.

Note: If the sample is in a buffer that contains primary amines (e.g., Tris or glycine), these compounds will quench the coupling reaction and must be thoroughly removed by dialysis or desalting.

Coupling Procedure

A. Column Preparation and Protein Immobilization

- For all steps requiring mixing the coupling gel with buffer or the sample, gently tap column near the pellet several times until it becomes lose and then gently swirl the column or briefly vortex at low speed.
- Ensure that the gel remains wet at all times.
- Unless otherwise indicated, perform centrifugation steps at 1,000 x *g* for 1 minute.

1. Equilibrate the AminoLink® Plus Coupling Gel Spin Column and reagents to room temperature.
2. Loosen the column top cap first and then remove the bottom plug to avoid drawing air into the column. Place column in a collection tube and centrifuge at 1,000 x *g* for 2 minutes.
3. Remove the column cap and insert plug. To resuspend gel, add 300 µl of Coupling Buffer or other buffer used to dissolve the sample. Remove plug, place column in a collection tube, centrifuge and discard flow-through. Repeat this step two more times.

   Note: Unless otherwise indicated, perform centrifugation steps at 1,000 x *g* for 1 minute.
4. Plug column and add 200-300 µl of sample (0.5-1.5 mg/ml) directly onto the gel. Replace cap and mix. To determine coupling efficiency, reserve a portion of the sample for use as the starting amount.

   Note: For all steps requiring mixing of the gel, swirl the column gently or briefly vortex at low speed.
5. In a fume hood, uncap column and add 2 µl of the Sodium Cyanoborohydride Solution to the reaction slurry. Replace cap and mix.
6. Incubate column at room temperature for 4 hours. Mix reaction every hour. Alternatively, incubate overnight at 4°C with gentle end-over-end mixing

   Note: For overnight incubation, use a microcentrifuge tube mixer for end-over-end mixing. Make sure the gel is adequately mixing. Adding a final concentration of 0.05% Tween®-20 will help the gel to flow freely in the column.
7. Loosen column cap and remove plug. Place column in a collection tube and centrifuge. To determine coupling efficiency, evaluate the flow-through by protein assay and compare to the starting amount.
8. Uncap column and insert plug. Add 300 µl of Coupling Buffer replace cap and mix. Place column in a collection tube and centrifuge. Repeat this step two more times. Reserve flow-through to evaluate coupling efficiency.

   Note: If the reaction was incubated overnight, then gently invert column 10 times to dislodge any gel remaining in the cap. Loosen column cap and remove plug. Place column in a collection tube and centrifuge. Reserve flow-through to evaluate coupling efficiency. Repeat this step two more times.

B. Block Remaining Active Binding Sites

1. Uncap column and insert plug. Add 300 µl of Quenching Buffer onto the gel replace cap and mix.
2. Remove column cap and plug. Place column in a collection tube, centrifuge and discard flow-through.
3. Repeat Steps 1-2.
4. Plug column and add 200 µl of Quenching Buffer directly onto the gel. Replace cap and mix.
5. In a fume hood, uncap column and add 4 µl of the Sodium Cyanoborohydride Solution to the reaction slurry. Cap column and mix. Incubate reaction at room temperature for 30 minutes mixing every 15 minutes.
6. Remove cap and plug. Place column in a collection tube, centrifuge and discard flow-through.

C. Wash and Store the Affinity Column

Note: For long-term storage (i.e., >2 weeks), add sodium azide to the Coupling Buffer at a final concentration of 0.02%.

1. Plug column and add 300 µl of Wash Solution. Replace cap and mix. Remove cap and plug, place column in a collection tube, centrifuge, and discard flow-through. Repeat this step two more times.
2. Plug column and add 300 µl of Coupling Buffer, replace cap and mix. Remove cap and bottom plug, place column in a collection tube. Centrifuge tube and discard flow-through. Repeat this step two more times.
3. Plug column and add 300 µl of Coupling Buffer along the sides of the column to wash down the gel. Replace cap and store at 4°C.

General Procedure for Affinity Purification

Note: Empirically determine the optimal amount of sample needed and the incubation time for the specific antibody-antigen system being used.

A. Form the Gel-bound Complex

1. Equilibrate the gel containing the immobilized antibody to room temperature.
2. Remove the column cap first and then the plug. Place column in a collection tube, centrifuge and discard flow-through.
3. Plug column and add 200-300 µl of the sample directly onto the gel. Replace cap and mix.
4. Incubate reaction with gentle end-over-end mixing or rocking. Typical incubations range from 2 hours at room temperature to overnight at 4°C.
5. Loosen column cap and remove plug. Place column in a collection tube and centrifuge. Discard or save flow-through for analysis.

   Note: Steps 3-5 may be repeated if additional sample is available and the column's capacity has not been exceeded. Column capacity must be determined empirically.
6. To reduce possible nonspecific interactions (optional) prepare 1 ml of 0.5 M NaCl (dilute the Wash Solution 1:1) containing a final concentration of 0.05% Tween®-20. Uncap column, insert plug and add 300 µl of the solution. Replace cap and gently invert column 10 times. Loosen cap, remove plug and place column in a collection tube. Centrifuge tube and discard flow-through. Repeat this step two additional times.
7. Uncap column and insert plug. Add 300 µl of Coupling Buffer, replace cap and gently invert tube 10 times. Loosen cap and remove plug. Place column in a collection tube, centrifuge and discard flow-through. Repeat this step once.
8. Uncap column and insert plug. Add 300 µl of Coupling Buffer, in increments of 100 µl, along sides of the column to wash down the gel. Do not mix the gel. Remove plug, place column in a collection tube, centrifuge and discard flow-through.

B. Elution

1. Plug column and add 100 µl of ImmunoPure® Elution Buffer along the sides of the column onto the gel. Replace cap and mix. Incubate column at room temperature for 10 minutes.

   Note: Effective elution conditions are dependent on the individual antibody-antigen system and may require optimization.

2. Uncap column and insert plug. Place column in a collection tube and centrifuge. Neutralize the low pH of the eluted fraction by adding 5 µl of 1 M Tris, pH 9.0 or immediately dialyze against PBS using a Slide-A-Lyzer® MINI Dialysis Unit (e.g., Product No. 69576).
3. Repeat Steps 1 and 2 as needed. See the Additional Information Section for a method to quickly evaluate elution.
4. Regenerate the gel as soon as possible after elution by washing three times with 300 µl of Coupling Buffer containing 0.02% sodium azide. Store column at 4°C.

Troubleshooting

Problem	Possible Cause	Solution
Low coupling efficiency	Primary amines not completely removed from sample before coupling	Completely remove primary amines by extensive dialysis or desalting
	Protein precipitated during coupling	Check the solubility of the protein in coupling buffer for 4 hours
Protein/peptide is not soluble in Coupling Buffer	Molecule is hydrophobic	Dissolve molecule in Coupling Buffer containing up to 4 M Guanidine•HCl or 20% organic solvent such as DMSO, DMF or CH_3CN
Antigen does not immunoprecipitate	Sample does not contain enough antigen to detect	Verify protein expression and/or lysis efficiency by SDS-PAGE or Western blot analysis of the lysate
	Antibody is not coupled to the gel	Verify that the antibody is coupled to the gel by determining the coupling efficiency
	Antibody is sensitive to low pH and has become inactive during elution	Prepare another antibody-coupled column and use a high-salt, neutral pH elution buffer such as ImmunoPure® Gentle Elution Buffer (Product No. 21027)
	The antibody-antigen interaction does not elute using acidic conditions	Use a high-salt neutral pH elution buffer, guanidine•HCl, urea, lithium bromide, potassium thiocyanate or nonionic detergents to elute antigen **Note:** Using denaturants may cause the antibody to lose activity and, therefore, the antibody-coupled gel cannot be reused

Additional Information

Quick Evaluation of Elution using MemCode™ Reversible Stain for Nitrocellulose Membranes (Product No. 24580)

1. Dot blot 5-10 µl of each elution fraction onto dry nitrocellulose membrane and let the proteins bind for 2-5 minutes.
2. Wash three times with ultrapure water.
3. Add 25 ml MemCode™ Protein Stain and shake at room temperature for 30 seconds.
4. Rinse three times with ultrapure water.

Related Pierce Products

69715 **Handee™ Microcentrifuge Tubes,** 72 each

28372 **BupH™ Phosphate Buffered Saline Pack,** 40 packs

21027 **ImmunoPure® Gentle Ag/Ab Elution Buffer,** 500 ml

21004 **ImmunoPure® IgG Elution Buffer,** 1 L

24580 **MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes**

88013 **Nitrocellulose Membrane, 0.2 µm,** 7.9 x 10.5 cm, 15 sheets/pkg

References

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The most current versions of all product instructions are available at *www.piercenet.com*. For a faxed copy, contact customer service (in the USA call 800-874-3723) or your local distributor.