INSTRUCTIONS

MicroLink™ Peptide Coupling Kit

20485

1510.3

Number	Description
20485	**MicroLink™ Peptide Coupling Kit,** contains sufficient reagents for 10 immobilizations, each with 25-250 µg of sulfhydryl-containing peptide or protein

Kit Contents:

UltraLink® Iodoacetyl Gel Spin Columns, 10 each (each column contains 400 µl of 25% slurry)

Coupling Buffer, 100 ml, contains 50 mM Tris, 5 mM EDTA; pH 8.5

L-Cysteine•HCl, 100 mg

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

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

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

Microcentrifuge Collection Tubes, 200 each

Storage: Upon receipt store product in the dark at 4°C. Product shipped at ambient temperature.

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Introduction

MicroLink™ Peptide Coupling Kit enables simple and efficient covalent immobilization of sulfhydryl-containing peptides, proteins and other ligands to a gel support for use in small-scale affinity purification procedures. This kit uses UltraLink® Iodoacetyl Gel that reacts specifically with free sulfhydryls to form a stable thioether linkage. The support contains a 15-atom spacer that reduces steric hindrance, making binding interactions with the coupled molecule efficient. Average coupling efficiencies are greater than 80%, although efficiency is related to sulfhydryl content and is variable. This kit is ideal for coupling small quantities of sulfhydryl-containing peptide for subsequent antibody purification.

Important Product Information

The peptide or protein to be immobilized must have free (reduced) sulfhydryls. Ellman's Reagent (Product No. 22582) can be used to determine the presence of free sulfhydryls. To cleave disulfide bonds to make sulfhydryls use a reducing agent, such as dithiothreitol (DTT, see Related Thermo Scientific Products), followed by desalting or dialysis to remove the reducing agent.

- Peptide samples: Most peptides are too small to be purified efficiently by desalting (gel filtration). In such cases, a convenient alternative is to reduce peptide disulfide bonds using Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). After a brief incubation with an equal volume of the Reducing Gel, the reduced peptide is easily separated from the Immobilized TCEP reagent by centrifugation.
- Protein samples: Reduce disulfide bonds in high molecular weight proteins using 5 mM TCEP (1:100 dilution of Bond-Breaker® TCEP Solution, Product No. 77720) for 30 minutes at room temperature, followed by two passes through an appropriate desalting column (e.g., Zeba™ Desalt Spin Columns, Product No. 89882). Be aware that proteins (e.g., antibodies) may be inactivated by complete reduction of disulfide bonds they contain. Selective reduction of hinge-region disulfide bonds in IgG may be accomplished with 2-Mercaptoethylamine•HCl (2-MEA, Product No. 20408).

Material Preparation

Peptide or Protein Sample	Dissolve 25-500 µg of sample in 200-300 µl of Coupling Buffer (0.1-1.5 mg/ml). If the sample is not soluble in Coupling Buffer, dissolve it in a suitable buffer at pH 8-8.5 (see the Troubleshooting Section). Dilute samples already in solution 1:1 in Coupling Buffer, but do not exceed 300 µl total. **Note:** If sample buffer contains sulfhydryls (e.g., 2-mercaptoethanol or DTT), these compounds will quench the coupling reaction and must be thoroughly removed by dialysis or desalting.
Phosphate Buffered Saline (PBS)	Dissolve the dry-blend buffer with 500 ml of ultrapure water. For long-term storage of excess buffer, sterile filter the solution and store at 4°C. A final concentration of 0.02% sodium azide may be added as a preservative.

Procedure for Peptide or Protein Coupling

A. Spin Column Preparation and Coupling

- For all steps requiring mixing the coupling gel with buffer or the sample, gently tap column near the pellet several times until it becomes loose and then gently swirl the column or briefly vortex at low speed.
- Ensure that the gel remains wet at all times.

1. Equilibrate the UltraLink® Iodoacetyl Gel Spin Columns and all reagents to room temperature.
2. Loosen the column's 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 × *g* for 2 minutes.
3. Remove the column cap and insert plug. To resuspend the 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 × *g* for 1 minute.
4. Plug column and add 200-300 µl of the 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. Incubate column at room temperature for 1 hour or overnight at 4°C with gentle end-over-end mixing. Alternatively, gently invert column 10 times every 15 minutes.

   Note: When using a microcentrifuge tube mixer for end-over-end mixing, make sure the reaction slurry is adequately mixing. Adding a final concentration of 0.05% Tween®-20 helps the gel slurry to flow freely in the column.
6. 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.

7. Uncap column and insert plug. Add 300 µl of Coupling Buffer and replace top cap. Gently invert column 10 times. Loosen column cap and remove plug. Place column in a collection tube and centrifuge. Repeat this step two more times. To determine coupling efficiency, evaluate the flow-through by protein assay.

B. Block Remaining Active Binding Sites

1. Uncap column and insert plug. Dissolve 2 mg of L-Cysteine•HCl in 200 µl of Coupling Buffer, add it to the gel and mix.
2. Incubate column at room temperature for 1 hour mixing every 15 minutes.
3. Uncap column and remove plug. Place column in a collection tube, centrifuge and discard flow-through.

C. Wash and Store the Affinity Column

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

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

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 immobilized peptide or protein 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. Cap column 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 binding capacity has not been exceeded. 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. Remove the column's top cap, insert bottom plug and add 300 µl of the solution. Replace top cap and gently invert column 10 times. Loosen top cap, remove bottom 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 PBS, replace cap and gently invert tube 10 times. Loosen column cap and remove plug. Place column in a collection tube, centrifuge and discard flow-through. Repeat this two additional times.
8. Uncap column and insert plug. Add 300 µl of PBS, 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 Elution Buffer along the sides of the column onto the gel. Cap column and mix. Incubate 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
Peptide or protein precipitates in Coupling Buffer	Peptide or protein is insoluble in Coupling Buffer	Dissolve sample in ≤ 30% DMSO or DMF or 6 M guanadine•HCl
Low coupling efficiency	Disulfide bond(s) are not reduced	Reduce disulfide bonds and proceed immediately with desalting and coupling procedure to minimize disulfide bonds reformation
	Reductant not removed from sample	Remove reductant from the reduced sample using a desalting column
Antibody or antigen does not immunoprecipitate	The ligand-receptor interaction does not elute using acidic conditions	Use a neutral pH elution buffer, guanidine•HCl, urea, lithium bromide, potassium thiocyanate or nonionic detergents to elute
Purified antibody does not function in the downstream application	Antibody is sensitive to the low-pH elution	Use a high-salt, neutral-pH elution buffer such as Gentle Elution Buffer (Product No. 21027)

Additional Information

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

1. Dot blot with 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 Thermo Scientific Products

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

20291 **No-Weigh™ Dithiothreitol (DTT),** 48 × 7.7 mg microtubes

77712 **Immobilized TCEP Disulfide Reducing Gel,** 5 ml

77720 **Bond-Breaker® TCEP Solution,** 5 ml

22582 **Ellman's Reagent,** 5 g

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

21004 **IgG Elution Buffer,** 1 L

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

20408 **2-Mercaptoethylamine•HCl,** 6 × 6 mg

General References for UltraLink Biosupport

Coleman, P.L., *et al.* (1988). Affinity chromatography on a novel support: azlactone-acrylamide copolymer beads. *FASEB J.* **2**:A1770 (#8563).

Hermanson, G.T., Mallia, A.K. and Smith, P.K. (1992) Immobilized Affinity Ligand Techniques. Academic Press, Inc., pp. 28-31, 90-95. (This book is available from our catalog and website as Product No. 22230.)

Product References for UltraLink Iodoacetyl Gel

Bicknell, Andrew B., *et al.* (2001). Characterization of a serine protease that cleaves pro-γ-melanotropin at the adrenal to stimulate growth. *Cell*.**105:**903-12.

Liu, L.A. and Engvall, E. (1999). Sarcoglycan isofoms skeletal muscle. *J. Biol. Chem.* **274(53):**38171-6.

Magdesian, M.H., *et al.* (2001). Infection by *Trypanosoma cruzi*. *J. Biol. Chem.* **276(22):**19382-9.

Slide-A-Lyzer® MINI Dialysis Unit Technology is protected by U.S. Patent # 6,039,781.

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.