INSTRUCTIONS

UltraLink® Iodoacetyl Gel

53155

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0542.1

Number	Description
53155	**UltraLink® Iodoacetyl Gel**
	Kit Includes:
	UltraLink® Iodoacetyl Gel, 10 ml
	Disposable Column Trial Pack, includes the following:
	Disposable Polystyrene Columns, 0.5-2.0 ml, 2 each
	Disposable Polypropylene Columns, 1.0-5.0 ml, 2 each
	Disposable Polypropylene Columns, 2.0-10.0 ml, 2 each
	Accessory Pack, 1 each
	Storage: Upon receipt product should be stored at 4°C, protected from light.
	Product is guaranteed for one year from the date of purchase when handled and stored properly.

Introduction

UltraLink® Iodoacetyl Gel binds specifically to sulfhydryls when used under the conditions outlined here. Sulfhydryls react faster than other groups with the iodoacetyl alkylating agent. If the molar ratio of the reagent to protein is limited to a small excess over the sulfhydryl content, the reaction is limited to the sulfhydryls.[1] The 15-atom spacer arm reduces steric hindrance that could be associated with a support without a spacer arm and makes binding more efficient. This longer spacer arm is especially ideal for conjugating small peptides to the support. The support will immobilize numerous types of molecules with a free sulfhydryl including antibodies, other proteins and peptides.

UltraLink® Iodoacetyl Gel is prepared on UltraLink® Biosupport Medium.[2-8] The porosity, rigidity and durability of this support are important considerations when working with large volumes of samples requiring fast-flow techniques and large-scale bulk applications. UltraLink® Biosupport Medium is useful for medium- pressure techniques with rapid throughput. The support has a maximum linear flow rate of 3,000 cm/hour.

Protocol for Coupling to UltraLink® Iodoacetyl Gel

Note: The UltraLink® Iodoacetyl Gel must be stored in the dark.

Note: If you are immobilizing a peptide with a terminal cysteine or free sulfhydryl you may proceed directly to Step B, Coupling to UltraLink® Iodoacetyl Gel. Ellman's Reagent (Product No. 22582) can be used to determine if there are free sulfhydryls available on the protein or the peptide.

A. Reduction of Protein

1. Dissolve or dilute 1-10 mg protein per ml in 0.1 M sodium phosphate, 5 mM EDTA-Na, pH 6.0.
2. Add 6 mg of 2-mercaptoethylamine per ml of protein solution.
3. Incubate mixture at 37°C for 1.5 hours.
4. Cool mixture to room temperature.

5. Apply mixture to a desalting column equilibrated with 50 mM Tris, 5 mM EDTA-Na, pH 8.5 to remove excess 2-mercaptoethylamine.

   Note: Sample cannot exceed one-quarter of the volume of the desalting column or separation of the reductant from the sample will be incomplete.

6. Collect 1 ml fractions and monitor protein at A_{280}.
7. Fractions containing the protein should be pooled.

B. Coupling to UltraLink® Iodoacetyl Gel

1. Bring UltraLink® Iodoacetyl Gel to room temperature.
2. Pack appropriate sized column for your application. A disposable column trial kit is supplied for your convenience. It may be advantageous to use a peristaltic pump to increase the flow through the column. The beads can withstand 100 psi.
3. Equilibrate column with 6 column volumes of 50 mM Tris, 5 mM EDTA-Na, pH 8.5.
4. Replace bottom cap.
5. Add sulfhydryl containing sample prepared above. If using a lyophilized peptide, dissolve it in 50 mM Tris, 5 mM EDTA-Na, pH 8.5. Approximately 1 ml of sample solution can be applied per ml of UltraLink® Iodoacetyl Gel.

   Note: Retain a small sample of the peptide or protein solution if you wish to determine coupling efficiency.

6. Replace the top cap and mix column at room temperature for 15 minutes.
7. Incubate column at room temperature for 30 minutes without mixing.
8. Drain buffer.
9. Wash column with 3 column volumes of 50 mM Tris, 5 mM EDTA-Na, pH 8.5.
10. Determine protein content of starting material and wash fractions to establish coupling efficiency. Coomassie® Plus Protein Assay (Product No. 23236) is recommended.

C. Blocking Nonspecific Binding Sites on Gel

1. Replace the bottom cap.
2. Make a 50 mM cysteine solution in 50 mM Tris, 5 mM EDTA-Na, pH 8.5.
3. Apply 1 ml of 50 mM cysteine to column per 1 ml of gel.
4. Replace the top cap.
5. Mix for 15 minutes at room temperature.
6. Incubate column at room temperature for 30 minutes without mixing.

D. Washing the Column

1. Remove top and bottom caps sequentially and drain the liquid.
2. Wash the column with 16 column volumes of 1 M NaCl and 16 column volumes of degassed 0.05% sodium azide, respectively.

E. Storage of the Coupled Column

1. Replace the bottom cap.
2. Add additional degassed 0.05% sodium azide.
3. Replace the top cap.
4. Store upright at 4°C.
5. The column is now ready to use.

Related Pierce Products

20408	**2-MEA-HCl (mercaptoethylamine)**
22582	**Ellman's Reagent**

23236	**Coomassie® Plus Protein Assay Reagent**
43426	**D-Salt™ Polyacrylamide Plastic Desalting Columns**, 1.8K MWCO, 5 x 5 ml
43230	**D-Salt™ Dextran Plastic Desalting Columns**, 5K MWCO, 5 x 5 ml
43443	**D-Salt™ Polyacrylamide Plastic Desalting Columns**, 6K MWCO, 5 x 10 ml
22230	**Immobilized Affinity Ligand Techniques,** book by Hermanson, G., *et al*. (1992). Academic Press, San Diego, CA (an excellent overview of Affinity Matrices)

Cited References

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Product References

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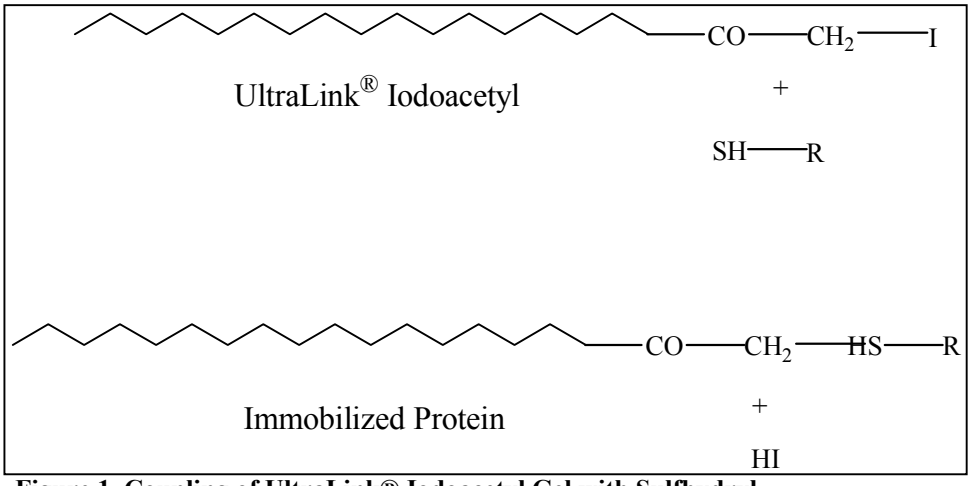

Figure 1. Coupling of UltraLink® Iodoacetyl Gel with Sulfhydryl.

US Patent 4,871,824
European Patent EPO 392,824