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A Geno Technology, Inc. (USA) brand name

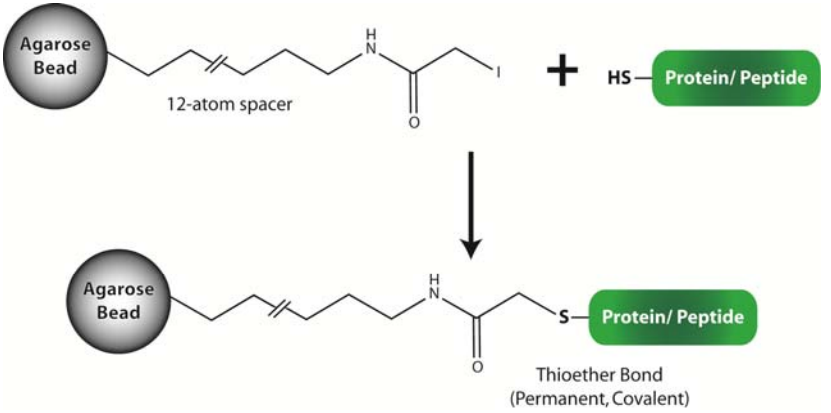
Sulfhydryl Coupling Resin

For Covalent Immobilization of Sulfhydryl Containing Proteins, Peptides & Ligands

(Cat. # 786-794, 786-795, 786-796, 786-806)

INTRODUCTION

The Sulfhydryl Coupling Resin is designed for the simple and efficient coupling of peptides and proteins to a solid agarose support through free sulfhydryl groups. The iodoacetyl groups of the Sulfhydryl Coupling Resin specifically react with free sulfhydryls to form covalent, permanent thioether bonds (see figure). The long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.



ITEM(S) SUPPLIED

Cat. #	Description	Size
786-794	Sulfhydryl Coupling Resin	10ml resin
786-795	Sulfhydryl Coupling Resin	50ml resin
786-796	Sulfhydryl Coupling Resin	250ml resin
786-806	Sulfhydryl Coupling Resin	5 x 2ml columns

STORAGE CONDITIONS

Shipped at ambient temperature. Upon receipt store at 4°C, do NOT freeze.

IMPORTANT

- **Activity:** 1mg sulfhydryl peptide/ml of resin, ~5mg reduced protein/ml resin
- **Support**: 6% Cross-linked Agarose

ADDITIONAL COMPONENTS REQUIRED

- Columns, glass or plastic. Choose a size applicable to the amount of resin used.
- Coupling Buffer (50mM Tris, 5mM EDTA, pH8.5); prepare > 20 column volumes.
- L-Cysteine•HCl
- 1M Sodium Chloride
- PBS with 0.05% sodium azide

PREPARATION BEFORE USE

1. The ligand to be coupled must contain a free (reduced) sulfhydryl group.
   a. To determine the presence of a free sulfhydryl, assay with Ellman's reagent (Cat. # BC87). See Appendix for assay protocol.
   b. To generate free sulfhydryls, reduce the proteins/peptides with a suitable reducing agent followed by desalting or dialysis. See Related Products for applicable products.

PROCEDURE FOR RESIN

1. Gently swirl the bottle of Sulfhydryl Coupling resin to achieve a homogenous suspension. Using a wide bore pipette transfer the resin slurry to an appropriate column. For every 1ml resin bed use 2ml 50% slurry.
   NOTE: *Throughout the procedure ensure the resin in the gravity flow columns does not become dry. If necessary add additional Coupling Buffer and cap the bottom of the column.*
2. Equilibrate the column with 4 column volumes of Coupling Buffer.
3. Prepare the reduced peptide/protein in Coupling Buffer and gently apply 1-2ml peptide/protein solution for every ml settled resin.
   OPTIONAL: *Retain a small amount of peptide/protein solution to determine the coupling efficiency*
4. Seal the column and incubate at room temperature for 15- 30minutes with tumbling or rocking.
5. Place the column and allow to settle by incubating for a further 10-15 minutes.
6. Remove the top then bottom cap and collect the flow through.
7. Wash the column with 3 column volumes of Coupling Buffer, discard the washes.
8. Determine the coupling efficiency by measuring and comparing the peptide/protein concentrations of the flow through (Step 6) with the starting material (Step 3).
9. Prior to use, prepare a 50mM L-Cysteine•HCl in Coupling Buffer and add one column volume to the capped column.
10. Mix for 30 minutes at room temperature and then incubate for a further 15 minutes without mixing.
11. Remove the top then bottom cap and discard the flow through.
12. Wash the column with 6-10 column volumes 1M sodium chloride.
13. Wash the column with 2-4 column volumes degassed PBS with 0.05% sodium azide.
14. The column can now be stored at 4°C.

PROCEDURE FOR SPIN COLUMNS

1. Briefly centrifuge the column at 1,000xg for 2 minutes to collect the resin. Remove the top cap and snap off the bottom tab. Transfer to a 15ml centrifuge tube and centrifuge at 1,000xg for 2 minutes to remove the storage buffer.
2. Equilibrate the column with 2ml Coupling Buffer. Apply the Coupling Buffer and then centrifuge at 1,000xg for 2 minutes. Discard the flow through. Repeat three more times.
3. Prepare the reduced peptide/protein in Coupling Buffer and gently apply up to 3ml peptide/protein solution.
   OPTIONAL: Retain a small amount of peptide/protein solution to determine the coupling efficiency
4. Seal the column and incubate at room temperature for 15-30minutes with tumbling or rocking.
5. Place the column and allow to settle by incubating for a further 10-15 minutes.
6. Remove the top then bottom cap and collect the flow through by centrifuging at 1,000xg for 2 minutes.
7. Wash the column with 2ml Coupling Buffer, discard the wash. Repeat two more times.
8. Determine the coupling efficiency by measuring and comparing the peptide/protein concentrations of the flow through (Step 6) with the starting material (Step 3).
9. Prior to use, prepare a 50mM L-Cysteine•HCl in Coupling Buffer and add 2ml to the capped column.
10. Mix for 30 minutes at room temperature and then incubate for a further 15 minutes without mixing.
11. Remove the top then bottom cap and collect the flow through by centrifuging at 1,000xg for 2 minutes.
12. Wash the column with 6-10 column volumes 1M sodium chloride.
13. Wash the column with 2-4 column volumes degassed PBS with 0.05% sodium azide.
14. The column can now be stored at 4°C.

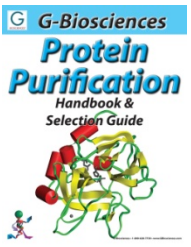
APPENDIX

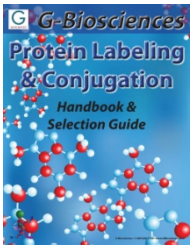
Ellman's Reagent (DTNB) Assay

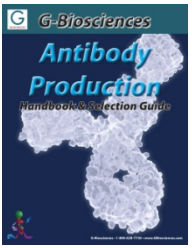
1. Make 10mM DTNB stock solution by dissolving 40mg DTNB in 10ml 0.1M Tris-HCl pH 7.5. The stock solution can be stored at 4°C for 3 months. Dilute the stock solution 100 fold with 0.1M Tris-HCl pH 7.5 to make 0.1mM DTNB working solution.
2. Aliquot 950□l of 0.1mM DTNB work solution to each 1.5ml centrifuge tube. Add 50□l test sample and mix by brief vortexing. Set a blank by adding 50□l of 0.1M Tris-HCl pH 7.5 to 950□l of 0.1mM DTNB work solution.
   ***NOTE**: The test sample may need to be diluted before applied to the assay and the dilution factor should be recorded. The 50µl test sample applied to the assay reaction should have a sulfhydryl concentration less than 0.5mM. Concentrations exceeding 0.5mM free sulfhydryl will result in high absorbance values and less accurate estimation of the concentration based on the extinction coefficient.*
3. Incubate 2 minutes at room temperature.
4. Measure the absorbance of the test sample with a spectrophotometer against blank at 412nm.
5. Calculate the concentration of free sulfhydryls in the sample from the molar extinction coefficient of NTB
   ($14.15\ mM^{-1}\ cm^{-1}$) as follow:
   mM free sulfhydryls = Absorbance / (path length x 14.15) x 20 x dilution factor
   Path length is the cuvette path length in centimeter (cm)
   20 is the dilution factor of 50µl sample to 1ml assay volume

RELATED PRODUCTS

Download our Protein Purification, Protein Labeling & Conjugation and Antibody Production Handbooks.







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