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# Sulfhydryl Immobilization Kit for Peptides

## For Generation of Peptide Affinity Columns Through Free Sulfhydryls

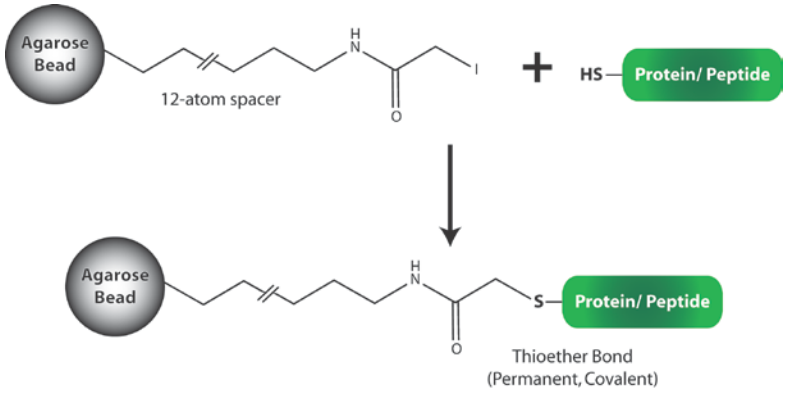
## (Cat. # 786-805)

## INTRODUCTION

The Sulfhydryl Immobilization Kit for Peptides is designed for the simple and efficient coupling of sulfhydryl-containing peptides to a solid agarose support. The Sulfhydryl Coupling Resin Columns utilizes iodoacetyl groups that 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.

Peptides must have free sulfhydryls for immobilization to the resin. The supplied Protein-S-S-Reductant™ reducing agent efficiently reduces disulfide bonds and does not interfere with the iodoacetyl coupling reaction. Protein-S-S-Reductant™ offers the advantage that it does not require removal before peptide immobilization.

The resulting columns can be used for the purification of antibodies that have been raised against the specific peptide. The columns, depending on the stability of the peptide, can be used several times without significant loss of activity.




## ITEMS SUPPLIED (Cat. #: 786-804)

| Part # | Description | Size |
|---|---|---|
| 480S-A | Sulfhydryl Coupling Columns | 5 x 2ml |
| 258O | Optimizer Buffer™ II [5X] | 25ml |
| 102W-A | Wash Solution | 60ml |
| 512P-B | Protein-S-S-Reductant™ | 1ml |
| 295C | L-Cysteine. HCl | 0.5g |

## STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival store at 4°C in the dark. Stable for 1 year.

## IMPORTANT INFORMATION

- Perform immobilization steps at room temperature. Equilibrate all reagents to room temperature before use.
- Maleimides react with free sulfhydryls to form stable thioether bonds at pH 6.5-7.5. pH >7.5 significantly increases the reaction of amines with the maleimide groups.
- Some sulfhydryl-containing peptides and proteins may oxidize in solution and form disulfide bonds, which cannot react with maleimides. Disulfide bonds can be reduced to produce free sulfhydryls. The G-Biosciences Immobilized Reductant (Cat. # 786-148) enable peptide or protein reduction while recovering the sample in the absence of reducing agents.
- Ellman's Reagent (Cat. # BC87) can be used to determine the amount of free sulfhydryls. (See Appendix)
- For peptides or proteins lacking sulfhydryls, SATA (N-Succinimidyl-S-acetylthioacetate) (Cat. # BC96) or Traut's Reagent (2-Iminothiolane hydrochloride) (Cat. # BC95) can be used to add sulfhydryls via amine modification. (See Appendix)
- Ensure the peptide solutions are free of particulates as these will inhibit column flow rates. We recommend filtering through a 0.45µm filter or a high speed centrifugation at 10,000g.
- Hydrophobic peptides may require additional washes or use of non-ionic detergent to minimize non-specific binding.
- Peptides with similar isoelectric point near Optimizer Buffer™ II (pH8.2) may not be completely solubilized. Hydrophobic peptides or other molecules that are not readily soluble in Optimizer Buffer™ II can be solubilized in water-miscible solvents up to 20%. Compatible solvent and solubilization components include 20% DMSO, 20% DMF, 20% ethanol, 1% Tween® 20 and 4 M urea.

## ADDITIONAL ITEMS REQUIRED

- 15ml centrifuge tubes
- 1X PBS with 0.05% sodium azide for column storage

## PREPARATION BEFORE USE

1. Prepare 1X Optimizer Buffer™ II (1ml 5X Optimizer Buffer™ in 4ml de-ionized water). Prepare 25ml 1X Optimizer Buffer™ II for each column preparation

## PROTOCOL

***A. Reduce Peptide***

1. Prepare 2ml 0.1-4mg/ml peptide solution in a 1X Optimizer Buffer™.
2. Allow Protein-S-S-Reductant™ to warm to room temperature and then add 0.1ml to the 2ml Peptide Solution.
3. Incubate at 37°C for 30 minutes with gentle mixing.

***B. Immobilize Peptide on Sulfhydryl Coupling Resin***

1. Invert the Sulfhydryl Coupling Columns 5-6 times to suspend the resin. Remove the top cap and then bottom tab and centrifuge for 1 minute at 1,000g in a 15ml centrifuge tube to remove the storage buffer.
2. Add 2ml 1X Optimizer Buffer™ II and centrifuge at 1,000g for 1 minute. Repeat this step once, then apply the bottom rubber stopper.
3. Slowly apply the reduced Peptide Solution to the Sulfhydryl Coupling Column. Apply the top cap and incubate at room temperature for 15 minutes with end-over-end mixing or rocking.
   ***NOTE****: Save 0.1ml Peptide Solution to determine coupling efficiency.*
4. Incubate the column upright for a further 30 minutes at room temperature without mixing.
5. After incubation, remove the top then bottom caps and collect the flow-through in a clean tube. Save the flow-through to determine the coupling efficiency.
   ***NOTE****: The coupling efficiency is determined by measuring and comparing the concentration of the peptide in Starting Material (Step 3) and in the flow-through (Step 5). The concentration may be determined by a suitable protein assay.*
6. Wash the column with at least 2ml Wash Solution and centrifuge at 1,000g for 1 minute. Repeat this wash step three more times.
7. Wash the column with 2ml 1X Optimizer Buffer™ II and centrifuge at 1,000g for 1 minute. Repeat this wash step once. Place the stopper on the bottom of the column.

***C. Block Non-Specific Sites***

1. Add 15.8mg L-Cysteine. HCl to 2ml 1X Optimizer Buffer™ II and slowly add to the column.
2. Apply the top cap and incubate at room temperature for 15 minutes with end-over-end mixing or rocking.
3. Incubate the column upright for a further 30 minutes at room temperature without mixing.
4. Remove the top then bottom caps and allow to drain. The column can now be stored or used for affinity purification.
5. For storage, wash the column with 2ml 1X PBS supplemented with 0.05% sodium azide. Repeat three times. Seal the bottom of the column and add 2ml 1X PBS supplemented with 0.05% sodium azide and apply the top cap. Store upright at 4°C.

## TROUBLESHOOTING

| Issue | Possible Cause | Suggested Resolution |
|---|---|---|
| Peptide precipitates in Optimizer Buffer™ II | Peptide insoluble | Dissolve peptide in a small amount of water miscible solvent and then add to Optimizer Buffer™ II. See Important Information |
| Low level of immobilization | Low or no free sulfhydryls | Immediately start coupling procedure after reducing agent treatment to prevent disulfide bonds reforming |
| Loss of binding capacity after several uses | Immobilized ligand altered by temperature, affinity purification conditions or time | Generate a new column |
| | Binding sites and pores blocked by non-specific binding or particulates | Filter samples through a 0.45µm filter or centrifuge at 10,000g to remove particulates |
| | | Use high salt concentrations, non-ionic detergents to reduce non-specific binding. |

## APPENDIX 1: GENERAL AFFINITY PURIFICATION PROTOCOL

The following protocol is a simple spin method that allows for rapid affinity purification. The columns can be used for gravity flow methods. The concentration of protein and binding conditions are dependent of the affinity interactions and will need to be optimized for each situation.

***Additional Components***

Binding/ Wash Buffer: PBS, TBS or other aqueous buffer that supports the desired affinity interaction. Degas the buffers to avoid introducing air bubbles.
Sample: Dissolved or exchanged in Binding/Wash Buffer
Elution Buffer: 0.1-0.2M Glycine•HCl at pH2.5-3.0
Neutralization Buffer: 1M Tris•HCl pH 8.5

***Procedure***

1. Allow the column and buffers to warm to room temperature.
2. Remove top then bottom caps and centrifuge the column at 1,000g for 1minute in a 15ml centrifuge tube. Discard the storage buffer.
3. Equilibrate the resin with 3 column volumes (CV) of Binding/Wash Buffer.
4. Add ≤2ml sample and allow the sample to enter the resin bed. Replace the bottom cap and then add 0.2ml Binding/Wash Buffer. Replace the top cap.
5. Incubate the column at room temperature with end-over-end mixing or rocking for 15-60 minutes.
6. Remove top then bottom caps and centrifuge the column at 1,000g for 1minute in a 15ml centrifuge tube. Add 1ml Binding/Wash Buffer and centrifuge again. Combine and save both flow-throughs to evaluate binding efficiency and capacity.
7. Wash the resin with 4CV of Binding/Wash Buffer.
8. Elute the protein with 1CV of Elution Buffer and collect in a centrifuge containing 100µl Neutralization Buffer. Repeat this step 2-3 times more. Samples can be used directly for SDS-PAGE or can be dialyzed or desalted for other downstream applications.
9. Equilibrate the column by adding 2CV Binding/Wash buffer and allow it to flow-through the resin.
10. Cap the bottom of the column and add 2CV Binding/Wash Buffer supplemented with 0.05% sodium azide for long term storage. Store upright at 4°C.

## APPENDIX 2: 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 950µl of 0.1mM DTNB work solution to each 1.5ml centrifuge tube. Add 50µl test sample and mix by brief vortexing. Set a blank by adding 50µl of 0.1M Tris-HCl pH 7.5 to 950µl 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*

## APPENDIX 3: USE OF SATA TO ADD SULFHYDRYLS

SATA (N-Succinimidyl S-Acetylthioacetate) (Cat. # BC96) introduce protected sulfhydryls into proteins, peptides and other molecules. It is a NHS esters of S-acetylthioacetic acid.

1. Immediately before reaction, dissolver ~7mg SATA in 0.5ml DMSO to give ~55mM solution.
2. Combine 1ml 2-10mg/ml protein solution in PBS with 10µl 55mM SATA.
3. Incubate at room temperature for 30 minutes
4. Desalt the solution with a desalting column equilibrated with PBS. We recommend G-Biosciences SpinOUT™ GT-600 (Cat. # 786-170).
5. Identify the fraction with the protein using absorbance at 280nm or a suitable assay.
6. Combine 1ml SATA-modified protein with 100µl 0.5M hydroxylamine, 25mM EDTA in PBS.
7. Incubate for 2 hours at room temperature.
8. Desalt as before using PBS supplemented with 10mM EDTA.

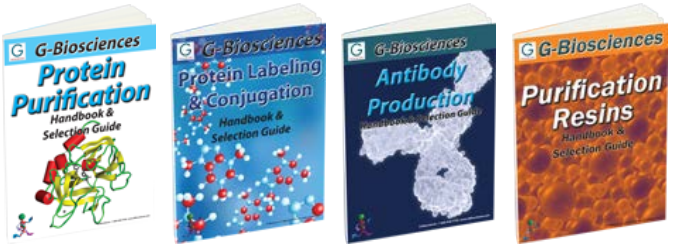
## APPENDIX 4: USE OF TRAUT'S REAGENT TO ADD SULFHYDRYLS

Traut's Reagent (2-Iminothiolane) (Cat. # BC95) is a cyclic thioimidate compound for thiolation of primary amines.

1. Dissolve the protein or peptide in a non-amine buffer at pH8.0. The addition of 2-5mM EDTA will prevent oxidation of generate sulfhydryls into disulfide bridges.
2. Add 2 to 20 fold molar excess of Traut's reagent to the protein solution.
   ***NOTE:*** *A 2mg/ml solution of Traut's reagent in water or buffer is a 14mM stock solution.*
3. Incubate the solution for 1 hour at room temperature.
4. Desalt the solution with a desalting column equilibrated with PBS with 2-5mM EDTA. We recommend G-Biosciences SpinOUT™ GT-600 (Cat. # 786-170).

## RELATED PRODUCTS

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

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