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A Geno Technology, Inc. (USA) brand name

Immobilized Reductant

(Cat. #786-148)

INTRODUCTION

Immobilized Reductant allows for a fast and reliable reduction of disulfide bridges in protein and peptide solutions.

Reducing agents are used in the reduction of disulfide bonds of proteins and peptides. Often it is necessary to remove the reducing agents from the protein/peptide solutions to prevent them interfering with subsequent procedures. For small proteins and particularly peptides it is almost impossible to remove the reducing agent from the protein/peptide using the standard practice of gel filtration, as the small proteins and peptides elute with the reducing agents. Immobilized Reductant is perfect for the reduction of small proteins and peptides as the reducing agent remains securely bound to the resin.

The Immobilized Reductant is supplied as 2ml resin in a spin column that can regenerated and reused for a total of five uses.

ITEMS SUPPLIED

Description	Size
Immobilized Reductant	2ml-resin

* Immobilized Reductant resin is supplied as a 50% slurry with 0.05% sodium azide as a preservative

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store refrigerated at 4°C, **DO NOT FREEZE**. This product is stable for 1 year at 4°C.

SPECIFICATIONS

Capacity: 15-25µmol sulfhydryl groups/ml resin
Bead Structure: 6% cross-linked agarose

ADDITIONAL ITEMS

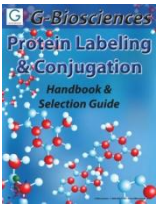
Equilibration Buffer: 0.1M Sodium phosphate, 1mM EDTA, pH8.0
Note: *To ensure all disulfide bonds are accessible to the Immobilized Reductant, a denaturing Equilibration Buffer can be used. Supplement the Equilibration Buffer with 6M guanidine•HCl. Use this to prepare protein/peptide solution and wash column with 5ml immediately prior to adding the protein/peptide solution.*
Activation Solution: 10mM DTT in Equilibration Buffer. Prepare by dissolving 15mg DTT in every 10ml Equilibration Buffer
Protein/ Peptide Sample: Dissolve 5-10mg peptide or ~5mg protein in 1ml Equilibration Buffer

PROCEDURE

1. Allow the column and Equilibration Buffer to warm to room temperature before use.
2. Briefly centrifuge the column in a 15ml centrifuge tube to collect the resin at the bottom of the column.
3. Remove the top and then bottom tab and allow the storage buffer to drain out of the column.
4. Apply 3ml Equilibration Buffer to the column and allow to drain through. Repeat this step two more times.
5. Prepare the Activation Solution by dissolving 15mg DTT in 10ml Equilibration Buffer.
6. Activate the column by applying a total volume of 9ml Activation Solution to the column. Apply as three 3ml aliquots.
7. Remove free Activation Solution by washing the column three times with 3ml Equilibration Buffer.
8. Apply 1ml peptide or protein solution to the column and allow it to completely enter the resin bed. Add 100µl Equilibration Buffer and then cap the top then bottom of the column and incubate the column at room temperature for 60 minutes for protein solutions. No incubation is required for peptide solutions.
9. Elute the reduced protein/peptide by applying nine 1ml aliquots of Equilibration Buffer. Collect separate 1ml fractions and analyze by reading the absorbance at 280nm.
   Note: *Some peptides may not adsorb significantly at 280nm. An alternative is to use Ellman's Reagent (Cat. # BC87) to detect the free sulfhydryls on the proteins or peptides.*
10. The column is ready to be reused. Simply follow the procedure from the beginning. For long term storage, wash the column with 5ml deionized water supplemented with 0.02% sodium azide. Store upright at 4°C.

RELATED PRODUCTS

Download our Protein Labeling & Conjugation Handbook.



http://info2.gbiosciences.com/complete-protein-cross-linkers-handbook

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