INSTRUCTIONS

Thermo SCIENTIFIC

Immobilized Reductant Columns

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77701

Number	Description
77701	**Immobilized Reductant Column**, pre-packed with 2mL Immobilized Reductant Resin Support: 6% crosslinked beaded agarose Capacity: 20-40µmol -SH groups per column per use **Storage:** Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific Immobilized Reductant Columns allow quick and convenient reduction of disulfide bonds in peptide and protein samples. Peptides are reduced efficiently in the few minutes required for the sample to pass through the gravity-flow column. This reduction is convenient because tedious and time-consuming gel filtration or HPLC steps are not required for subsequent separation of reducing agent from the sample. Instead, the proprietary reducing agent remains attached to the beaded agarose support and does not contaminate the recovered sample. This feature is especially important when treating peptides, whose small size prevents them from being desalted effectively by standard gel filtration.

Sulfhydryl (-SH) groups that occur in the side chain of cysteine residues in peptides and proteins are excellent targets for conjugation reactions. Applications for such conjugation reactions include crosslinking peptides to carrier proteins for use as immunogens and immobilization to solid supports for affinity purification of antibodies or binding partners. However, cysteine-containing peptides tend to dimerize in solution through formation of disulfide bonds, making the sulfur atoms unavailable for conjugation; disulfide bonds must be reduced to make sulfhydryls available for conjugation.

Although reduction of disulfide bonds in peptides and proteins can be performed with either dithiothreitol (DTT) or 2-mercaptoethanol (2-ME), these reducing agents must be removed by gel filtration before sulfhydryl-directed conjugation reactions can be performed. This extra step to remove the reducing agent can be time-consuming (allowing disulfide bonds to form again) and may result in loss or dilution of the sample peptide or protein.

Thermo Scientific Immobilized Reductant Columns circumvent these processing difficulties to enable rapid and efficient preparation of reduced samples for applications that cannot also contain reducing agent. Each column may be regenerated four times, for a total of five uses.

Additional Materials Required

- Equilibration Buffer #1: The optimal buffer for the reducing reaction is 0.1M sodium phosphate, 1mM EDTA, pH 8.0. Other buffers, such as phosphate-buffered saline (PBS), Tris-buffered saline (TBS) or HEPES may also be used.
- Equilibration Buffer #2 (optional): 0.1M sodium phosphate, 1mM EDTA, 6M guanidine•HCl, pH 8.0. This or similar buffer containing denaturant will help to expose protein disulfide bonds to the reducing agent and so make complete reduction of proteins more efficient.
- Dithiothreitol (DTT) Activation Solution: See related Pierce Products. For each use of a Reductant Column, a 10mM DTT solution in Equilibration Buffer #1 will be needed (15mg DTT dissolved in 10mL Equilibration Buffer #1). This solution must be prepared immediately before use (Step 4 of procedure).
- Protein or peptide sample: Dissolve 5-10mg peptide or 5-6mg protein in 1mL of Equilibration Buffer #1 or #2.

Procedure for Peptide or Protein Disulfide Reduction

1. Equilibrate Immobilized Reductant Column and prepared Equilibration Buffers (hereafter "Buffers") to room temperature before use. Perform all steps of this procedure at room temperature.
2. Remove top cap from the Immobilized Reductant Column and pour off storage solution, which contains 0.02% sodium azide.

   Note: When uncapping a column, always remove the top cap first to avoid drawing air into the resin bed. If the top porous disc in the column is dislodged, push it down to the resin bed surface (but avoid compressing the resin bed itself).
3. Twist off the reusable bottom closure and stand the column upright. SAVE the reusable bottom closure. When inverted, the bottom closure can be used to cap the column bottom.
4. Equilibrate/wash the Immobilized Reductant by adding 5mL of Buffer #1 to the column and allowing it to drain through.
5. Prepare DTT Activation Solution: Dissolve 15mg of DTT in 10mL Buffer #1 (results in 10mM DTT).
6. Activate the column by applying 10mL of DTT Activation Solution to the column and allowing it to drain through.
7. To remove the activating DTT, wash the column with 10mL of Buffer #1. (If Buffer #2 was chosen and used for the sample, wash column with only 5mL of Buffer #1, followed by 5mL of Buffer #2.)
8. Apply 1mL of peptide or protein solution to the column. Allow the sample to completely enter the resin bed. (Flow will stop automatically when the liquid drains down to the top porous disc.)
9. Cap the top and bottom of the column and incubate for 60 minutes for protein samples; no incubation is needed for peptide samples. To reseal the column, invert the snap-off closure from the column bottom (step 3), and with a slight twisting motion, press it firmly to the bottom tip of the column.
10. Recover reduced peptide or protein sample from the column by applying 9mL of the appropriate Buffer and collecting separate 1mL fractions as they emerge from the column tip. To ensure that the volume of each fraction is exactly 1mL, apply only 1mL of buffer at a time and collect the entire volume that emerges until column flow stops; then change collection tubes and apply the next 1mL of buffer. This method utilizes the stop-flow feature of the top porous disc.
11. Identify the fractions that contain the peptide or protein (now reduced) by determining which ones have peak absorbance at 280nm (usually the first two fractions). Be aware that some peptides do not absorb significantly at 280nm and cannot be assayed by this method. Alternatively, identify fractions that contain the reduced peptide or protein by specifically measuring for sulfhydryl groups using the Ellman's Reagent (Product No. 22582, see Related Thermo Scientific Products).

Column Regeneration and Storage

Reuse the Immobilized Reductant Column by simply repeating the procedure from the beginning; the DTT activation step will regenerate the column. Four cycles of regeneration (5 total uses) are possible with each Immobilized Reductant Column. For prolonged storage of used columns, first wash them with phosphate buffer or water containing 0.02% sodium azide, then replace top and bottom caps and store the columns upright at 4°C.

Related Thermo Scientific Products

20291	**Dithiothreitol (DTT), No-Weigh™ Format,** 7.7mg × 48 microtubes
20290	**Dithiothreitol (DTT),** 5g
22582	**Ellman's Reagent**, 5g
44899	**CarboxyLink™ Immobilization Kit,** 2mL
53155	**UltraLink™ Iodoacetyl Resin**, 10mL
21901	**EZ-Link™ Maleimide-PEG$_2$-Biotin**, 50mg
77712	**Immobilized TCEP Disulfide Reducing Gel**, 5mL

Product References

Stimmel, J.B., *et al.* (2000). Site-specific conjugation on serine-cysteine variant monoclonal antibodies. *J Biol Chem* **275(39):**30445-50.

Corse, E. and Machamer, C.E. (2000). Infectious bronchitis virus E protein is targeted to the golgi complex and directs release of virus-like particles. *J Virol* **74(9):** 4319-26.

Segura-Totten, M, *et al.* (2002). Barrier-to-autointegration factor: major roles in chromatin decondensation and nuclear assembly. *J Cell Biol* **158:** 475-85.

Al-Hallaq, R.A., *et al.* (2002). Association of NR3A with the N-methyl-D-aspartate receptor NR1 and NR2 subunits. *Mol Pharmacol* **62(5):** 1119-27.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition. The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0011211 B

Revision	Date	Description
B	31 July 2024	Correcting spin column usage.
A	17 October 2015	New document for Immobilized Reductant Columns.