INSTRUCTIONS

Thermo SCIENTIFIC

Pierce® CDI-Trisacryl

20259 20377

0097.4

Number	Description
20259	**Pierce CDI-Agarose,** 10mL of CDI activated 6% crosslinked beaded agarose
20377	**Pierce CDI-Trisacryl,** 50mL of CDI activated Trisacryl GF-2000

Resin supports are supplied as 50% slurries in acetone.

Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce CDI Resins (Table 1) are activated with 1,1´-carbonyl diimidazole (CDI) to form reactive imidazole carbamates. This reactive group is formed on the resin in organic solvent and stored as a suspension in acetone to prevent hydrolysis. Reaction of the resin in an aqueous coupling buffer with primary amine-containing ligands causes loss of the imidazole groups and formation of carbamate linkages (Figure 1). The coupling process occurs at basic pH (8.5-10). While having slower reaction with proteins than reductive amination (Thermo Scientific AminoLink Coupling Gel, Product No. 20281) or azlactone (Thermo Scientific UltraLink Biosupport, Product No. 53110), CDI resins are particularly adept at immobilizing peptides, small organic molecules and certain proteins. The reaction also can be performed in organic solvent to permit coupling of water-insoluble ligands.

Table 1. Characteristics of the CDI resins.

	Agarose	Trisacryl GF-2000
Bead size:	45-165µm	40-80µm
Fractionating range:	10,000-400,000 MW	10,000-1,000,000 MW
Activation level:	> 50µmol/mL gel	> 50µmol/mL gel

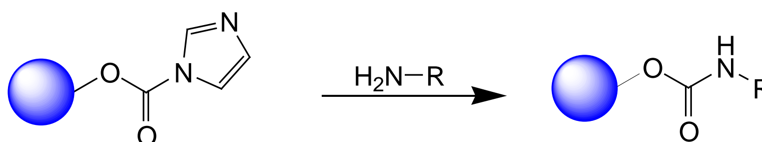

Figure 1. Reaction scheme of primary amines with Pierce CDI Supports.

Pierce Biotechnology
3747 N. Meridian Road
PO Box 117
Rockford, IL 61105 USA
(815) 968-0747
(815) 968-7316 fax
www.thermoscientific.com/pierce

Procedure for Coupling Proteins to Pierce CDI Supports

1. Dissolve protein to be coupled in 10-100mM borate or carbonate buffer, pH 8.5-11. Do not use buffers that contain primary amines (e.g., do not use Tris buffer). Use 2-10mg of protein per mL of resin to be used, and dissolve the protein in a volume approximately equal to the volume of resin bed.
2. Equilibrate the bottle of resin to room temperature before opening. The reactive group is moisture sensitive; equilibrating the product to room temperature ensures that moisture will not condense on the product and decrease its activity level for later use.
3. Add the resin to a Büchner funnel containing Whatman No.1 filter paper or sintered glass to remove acetone by washing with ice cold water. Do not allow resin to dry completely.
4. Combine washed resin and the protein solution (2-10mg of protein/mL of resin).
5. Incubate reaction slurry at room temperature for 2 hours (at pH 11) to overnight (at pH 8.5-10).

   Note: When using reaction condition below pH 10, extending the reaction time to 24 hours or longer will improve coupling efficiency.
6. Drain or otherwise remove excess coupling buffer from the reaction slurry.

   Note: The efficiency of coupling may be determined by comparing the protein concentration before and after coupling using the Thermo Scientific Pierce BCA Protein Assay (Product No. 23225) or Coomassie Plus™ – (Bradford) Assay Kit (Product No. 23236).
7. Add 50mM Tris buffer (pH 9-11) to the resin and incubate for several hours to quench and block nonreacted CDI groups.
8. Drain the Tris quenching buffer and wash the resin with phosphate (PBS) or other buffer.
9. Pack the coupled resin into a column or other device for use in affinity purification.

General References

Bethell, G.S. et. al. (1979). A novel method of activation of cross-linked agaroses with 1,1'-carbonyldiimidazole which gives a matrix for affinity chromatography devoid of additional charged groups. *J Biol Chem* 254, 2572-74.

Hearn, M.T.W. 1,1'-Carbonyldiimidazole-mediated immobilzation of enzymes and affinity ligands. In: Methods in Enyzmology, volume 135, pp. 102-17. (1987). Academic Press, Inc., San Diego, CA, USA.

Hermanson, G.T., Mallia, A.K. and Smith, P.K. In: Immobilized Affiity Ligand Techniques, pp. 64-69.(1992) Academic Press, Inc., San Diego, CA, USA.

Product References

Bethell, G.S. and Ayers, J.S. (1981). Investigation of the activation of various insoluble polysaccharides with 1,1'-Carbonyldiimidzaole and of the properties of the activated matrices. *J Chromatogr* 219, 361-72.

Cook, J.H., *et al.* (1984). Turnover and orientation of the major neural retina cell surface protein protected from tryptic cleavage by calcium. *Biochemistry* 23, 899-904.

Eaton, M.J., *et al*. (1990). Immunochemical characterization of brain synaptic membrane glutamate-binding proteins. *J Biol Chem* 265(27), 16195-204.

Edens, R.E., *et al.* (1990). Immobilization of C3b with retention of functional activity. *J Immunol Methods* 133(1), 67-70.

Hamada, N., *et al.* (1985). Identification of a thyroid microsomal antigen by Western blot and immunoprecipitation. *J Clin Endocrinol Metab* 61(1), 120-28.

Hearn, M.T.W., (1979). Application of 1,1'-Carbonyldiimidazole-activated agarose for the purification of proteins. II. The use of an activated matrix devoid of additional charged groups for the purification of thyroid proteins. *J Chromatogr* 185, 463-70.

Hissey, P.H., *et al.* (1985). Single-step monoclonal antibody affinity purification of human urogastrone from urine. *J Immunol Methods* 78, 211-16.

Jagoda, E.M., *et al.* (1994). An affinity column method for determination of the immunoreactivity of 131I-chimeric L6 monoclonal antibody and comparison to *in vivo* tumor localization. *J Immunol Methods* 173(2), 191-201.

Jonas, H.A., *et al.* (1986). An atypical insulin receptor with high affinity for insulin-like growth factors copurified with placental insulin receptors. *Proc Anal Acad Sci USA* 83, 4124-28.

Parra-Diaz, D., *et al.* (1993). A method for immobilzation of lipoxygenase. *Biotechnol Appl Biochem* 18, 359-67.

Portmann, L, *et al.* (1985). Anti-thyroid peroxidase antibody in patients with autoimmune thyroid disease: possible identity with anti-microsomal antibody. *J Clin Endocrinol Metab* 61(5), 1001-03.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2011 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.