G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Carboxyl Coupling Resin

High Capacity EAH Agarose/Immobilized DADPA (Diaminodipropylamine) For Generating Affinity Columns through Carboxyl Groups

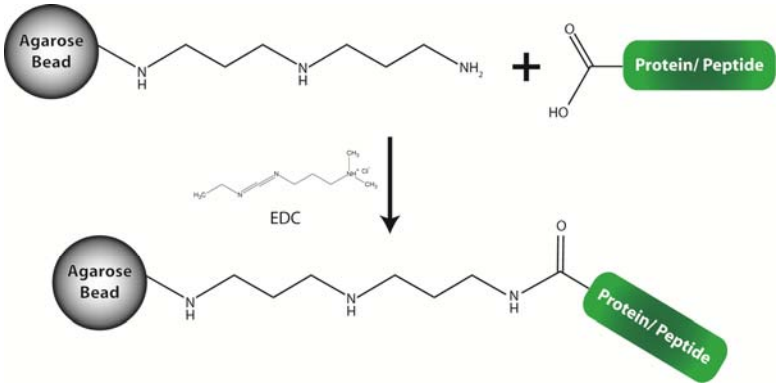
(Cat. #786-797)

INTRODUCTION

The Carboxyl Coupling Resin consists of 6% cross-linked agarose with covalent linked diaminodipropylamine (DADPA) to generate a free primary amine at the end of a long spacer arm (23Å). Molecules, including proteins and peptides, are covalently coupled to the free primary amines, and the stable columns are ideal for affinity purification of antibodies and other interacting partners.

Molecules can be coupled to the free amine by numerous amine-reactive methods; however the use of the carbodiimide EDC allows coupling of free carboxyl groups. The resulting amide bond is highly stable and greatly reduces the chance of leaching of the affinity tag. The 23Å long spacer arm reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification.

The Carboxyl Coupling Resin has a very high capacity of immobilized DADPA and offers >60µmole amine load for every ml of resin. This high capacity offers unsurpassed efficiency and capacity in the immobilization of carboxyl containing molecules.



ITEM(S) SUPPLIED (Cat. # 786-797)

Cat. #	Description	Size
786-797	Carboxyl Coupling Resin (Immobilized DADPA (Diaminodipropylamine))	25ml resin

** Immobilized Carboxyl Coupling Resin is supplied as a 50% slurry with 20% ethanol as a preservative.*

STORAGE CONDITION

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

IMPORTANT INFORMATION

- **Activity:** >60µmole amine/ml resin
- **Support**: 6% Cross-linked Agarose

PROTOCOL 1: IMMOBILIZING PEPTIDES/PROTEINS WITH EDC

Item(s) Required

- Columns, glass or plastic. Choose a size applicable to the amount of resin used. See Related Product for available columns.
- Coupling Buffer (0.1M MES Buffer, 0.9% NaCl)
  NOTE: For coupling reactions using EDC avoid the use of buffers containing free amines or phosphates as these will interfere with coupling efficiency. Tris, acetate and glycine buffers all readily react with EDC or the coupling intermediate. Thiol containing buffers should also be avoided as these irreversibly bind EDC and inhibit coupling.
- EDC; 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Cat. # BC25-1, BC25-5)
  NOTE: Allow the EDC to equilibrate to room temperature before opening to avoid condensation forming as it is moisture sensitive.
- 1M Sodium Chloride
- PBS with 0.05% sodium azide

Preparation Before Use

Peptide Preparation: EDC will react with peptide C-termini and side chains of aspartic and glutamic acid amino acids. In addition, EDC will react with the N-terminus of peptides resulting in peptide polymerization; however this usually does not affect affinity purification.

1. Dissolve the 1-10mg peptide/protein in 2ml Coupling Buffer.
   NOTE: For water insoluble peptides first resuspend in ethanol, methanol, DMSO or DMF and then add the solution to the Coupling Buffer, ensuring the solvent volume is less than 50% the total volume.

Procedure

1. Gently swirl the bottle of Carboxyl 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 5 column volumes of Coupling Buffer.
3. 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 5minutes with tumbling or rocking.
5. Immediately prior to use, add 0.5ml Coupling Buffer to 60mg EDC and then quickly transfer 0.5ml EDC solution to the column.

6. Seal the column and incubate at room temperature for 3 hours with tumbling or rocking.
7. Place the column and allow to settle by incubating for a further 10-15 minutes.
8. Remove the top then bottom cap and collect the flow through. Next add 1 column volume of 1M Sodium Chloride and collect with the flow through. This represents your unbound peptide and can be compared with your starting sample to determine coupling efficiency.
9. Wash the column with 3 column volumes of Coupling Buffer, discard the washes.
10. Wash the column with 2-4 column volumes degassed PBS with 0.05% sodium azide.
11. The column can now be stored at 4°C.

PROTOCOL 2: IMMOBILIZING NUCLEIC ACIDS/ OLIGONUCLEOTIDES THROUGH 5'-PHOSPHATE GROUPS

Item(s) Required

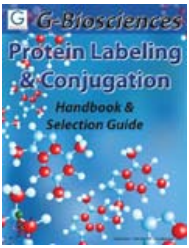
- Coupling Buffer (0.1M Imidazole, pH6)
- RNase/DNase free water. We recommend our Molecular Grade Water (Cat. # 786-72C)
- EDC; 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Cat. # BC25-1, BC25-5)
  NOTE: Allow the EDC to equilibrate to room temperature before opening to avoid condensation forming as it is moisture sensitive.

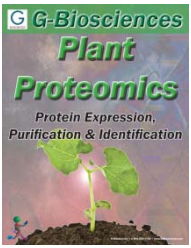
Procedure

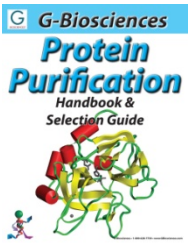
1. Gently swirl the bottle of Carboxyl Coupling resin to achieve a homogenous suspension. Using a wide bore pipette transfer the resin slurry to a microcentrifuge tube. For every 10µg oligonucleotide coupling use 1µl resin (2µl homogenous slurry).
2. Centrifuge for 2 minutes at 1,000xg. Carefully remove and discard the supernatant.
3. Wash the resin 3-5 times with 2 resin volumes of RNase/DNase free water by centrifuging for 2 minutes at 1,000xg and then carefully removing and discarding the wash.
4. Dissolve 10µg DNA or RNA in 1µl Coupling Buffer for every 1µl resin used.
5. Add the nucleic acid solution to the resin and mix well.
6. Immediately prior to use, add 67µl Coupling Buffer to 1mg EDC and then quickly transfer 2µl EDC solution to the resin for each µl of resin.
7. Incubate at room temperature for 3 hours with tumbling or rocking.
8. Centrifuge for 2 minutes at 1,000xg. Carefully remove the supernatant, which contains unbound nucleic acids/ oligonucleotides.
9. Wash the resin3-5 times with 2 resin volumes of RNase/DNase free water, or appropriate wash buffer (i.e. TE Buffer) by centrifuging for 2 minutes at 1,000xg and then carefully removing and discarding the wash.
10. Wash the column with 2-4 column volumes degassed PBS with 0.05% sodium azide.
11. The column can now be stored at 4°C.

RELATED PRODUCTS

Download our Handbook Labeling Protein Labeling and Conjugation.







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