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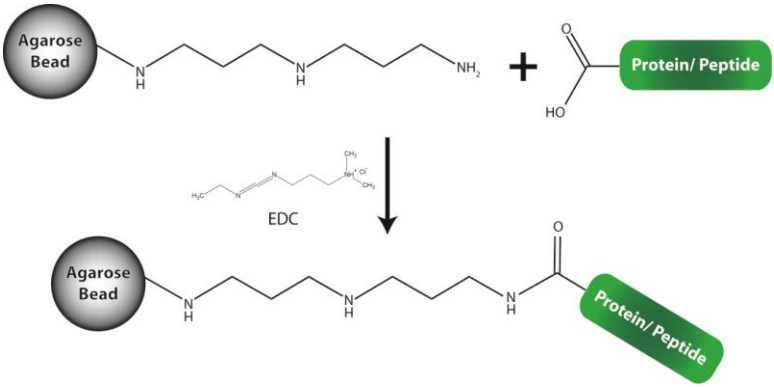
A Geno Technology, Inc. (USA) brand name

Carboxyl Immobilization Kit

Complete Kit For Generating Affinity Columns through Carboxyl Groups. Using High Capacity Immobilized DADPA (Diaminodipropylamine)

(Cat. #786-809)

INTRODUCTION



G-Biosciences Carboxyl Immobilization kit utilizes the Carboxyl Coupling Resin that 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 Carboxyl Immobilization kit uses the carbodiimide EDC to couple free carboxyl groups to the Carboxyl Coupling Resin. 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. The Carboxyl Immobilization kit can also be used for the immobilization of nucleic acids/ oligonucleotides through 5' phosphate groups.

ITEM(S) SUPPLIES (Cat. # 786-809)

Part. #	***Description***	***Size***
035C-A	*Carboxyl Coupling Resin Columns*	*5 x 2ml*
009E-C	*EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide]*	*5 x 50mg*
264O	*Optimizer Buffer™ IV [5X]*	*25ml*
102W-A	*Wash Solution*	*60ml*

** Immobilized Carboxyl Coupling Resin is supplied as a 50% slurry with 0.05% sodium azide as a preservative.*

STORAGE CONDITIONS

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

Additional Components

- *PBS with 0.05% sodium azide*

PREPARATION BEFORE USE

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

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 1X Optimizer Buffer™ IV.*
2. *NOTE: For water insoluble peptides first resuspend in ethanol, methanol, DMSO or DMF and then add the solution to the 1X Optimizer Buffer™ IV, ensuring the solvent volume is less than 50% the total volume.*
3. *Equilibrate the column, a vial of EDC and the buffers to room temperature*
4. *Remove the top cap and then twist off the bottom tab and allow the storage buffer to drain from the column.*
5. *Equilibrate the resin with 10ml 1X Optimizer Buffer™ IV and allow to pass though by gravity flow.*
6. *Seal the bottom of the column with a plastic stopper and then gently apply 2ml peptide/protein solution to the resin bed.*
7. *OPTIONAL: Retain a small amount of peptide/protein solution to determine the coupling efficiency.*
8. *Seal the column and incubate at room temperature for 5minutes with tumbling or rocking.*
9. *Immediately prior to use, add 0.5ml 1X Optimizer Buffer™ IV to the EDC vial and then quickly transfer the 0.5ml EDC solution to the column.*
10. *Seal the column and incubate at room temperature for 3 hours with tumbling or rocking.*
11. *Place the column and allow to settle by incubating for a further 10-15 minutes.*
12. *Remove the top then bottom cap and collect the flow through. Next add 2ml Wash Solution and collect with the flow through. The combined flow throughs represents*

your unbound peptide and can be compared with your starting sample to determine coupling efficiency.

13. *Wash the column with 8-10ml Wash Solution, discard the washes.*
14. *Wash the column with 4-8ml degassed PBS with 0.05% sodium azide.*
15. *The column can now be stored at 4°C.*

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

Additional Components

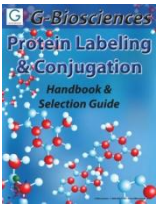
- *Coupling Buffer (0.1M Imidazole, pH6)*
- *RNase/DNase free water. We recommend our Molecular Grade Water (Cat. # 786-72C)*

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 Protein Labeling & Conjugation Handbook.



http://info2.gbiosciences.com/complete-protein-labeling-conjugation-handbook

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