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A Geno Technology, Inc. (USA) brand name

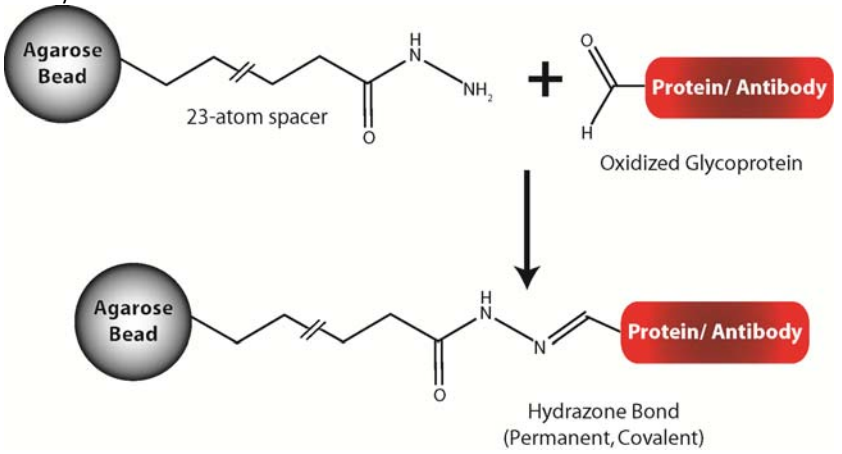
Carbohydrate Coupling Resin

For Covalent Immobilization of Glycoproteins to Agarose Resin

(Cat. #786-808)

INTRODUCTION

The Carbohydrate Coupling Resin is designed for the simple and efficient coupling of glycoproteins to a solid agarose support through oxidized sugar groups. The resin is ideal for immobilizing polyclonal antibodies as these are abundant in carbohydrates in their Fc domain. Their location in the Fc domain ensures the antibody's binding site is orientated away from the resin for optimal binding and reduced steric hindrance. Glycoprotein sugar components are first oxidized with sodium *meta*-periodate to convert the *cis*-glycol groups to reactive aldehydes. These aldehydes react spontaneously with the hydrazide group on the Carbohydrate Coupling Resin, forming stable hydrazone bonds (see figure). The long spacer arm (23Å) reduces steric hindrance and ensures greater binding of proteins and antibodies during affinity purification. The generated affinity resin can be reused at least 10 times with no significant loss of activity.



ITEM(S) SUPPLIED (Cat. # 786-808)

Part #	Description	Size
034C-A	Carbohydrate Coupling Resin	10ml

**Supplied as a 50% slurry in 20% ethanol*

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival store at 4˚C, do NOT freeze.

IMPORTANT

- **Activity:** 1-5mg oxidized polyclonal antibody or glycoprotein/ml of resin
- **Support:** 6% Cross-linked Agarose

ADDITIONAL ITEMS REQUIRED

- Columns, glass or plastic. Choose a size applicable to the amount of resin used.
- Coupling Buffer (0.1M Sodium acetate, 0.15M sodium chloride, pH 5.5); other buffers can be used, but avoid buffers with primary amines or sugars.
- Oxidizing agent: Sodium *meta*-periodate (Cat. # BKC-15)
- Desalting Columns, we recommend our SpinOUT™ GT-600 Desalting Columns.
- Wash Solution: 1M Sodium Chloride
- Optional: Aniline (CAS #: 62-53-3), a catalyst for improved coupling efficiency.
- PBS with 0.05% sodium azide

PREPARATION BEFORE USE

The glycoprotein to be coupled should be lyophilized or dissolved in an amine and sugar free aqueous buffer. If in an incompatible buffer dialyze or desalt against Coupling Buffer.

PROTOCOL

A. Sample Preparation

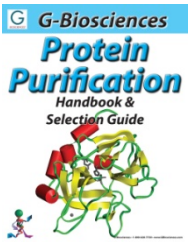
1. Dissolve 0.5-10mg glycoprotein (i.e. polyclonal antibody) in 1ml Coupling Buffer.
2. Weigh 2.5-5mg of Sodium *meta*-periodate into a small amber vial. This produces between ~11.5 and 23mM of oxidizing agent when dissolved in 1ml glycoprotein solution.
   NOTE: *The use of an amber vial is required as the oxidization reaction is light sensitive. If an amber vial is not available, seal an appropriate vial in aluminum foil.*
3. Add the 1ml glycoprotein solution to the vial of periodate and swirl gently to dissolve.
4. Incubate at room temperature for 30 minutes. Do not exceed 30 minutes or over oxidation may occur.
5. During the incubation, equilibrate a 5ml desalting column (Cat. # 786-704) with 15ml Coupling Buffer by applying the desalting resin and allowing it to pass through under gravity.
6. Slowly apply the oxidized glycoprotein to the desalting column and allow the solution to enter the resin bed.
7. Add 0.5ml Coupling Buffer to the desalting column and allow to enter the resin bed.
8. Centrifuge at 1,000g for 2 minutes to collect the oxidized protein.
9. **OPTIONAL:** In a fume hood, prepare 0.2M Carbohydrate Coupling Catalyst. Add 18µl aniline to 1ml Coupling Buffer. Vortex the catalyst for 15 seconds and add total volume to the oxidized glycoprotein sample. The final concentration in 0.1M aniline. Save 0.1ml to determine the coupling efficiency, if desired.

B. Couple to Carbohydrate Coupling Resin

1. Gently swirl the resin and pipette 4ml homogenous slurry (2ml resin) into a suitable columns. We recommend our 5ml Spin column (Cat. # 786-726).
2. Drain the storage buffer from the column and equilibrate with 5 resin volumes of Coupling Buffer. Allow the buffer to pass through the column by gravity flow. **NOTE:** Do not allow the resin to dry out during the coupling procedure.
3. Apply the bottom cap to the column and apply the 1ml oxidized glycoprotein to the resin. Seal with the top cap.
4. Incubate at room temperature with gently end-over-end mixing for 6 hours. The incubation can be extended to overnight. Avoid vigorous mixing as this may result in protein aggregation and precipitation.
5. After incubation, stand the column upright and allow the resin to settle for 15 minutes.
6. Remove the top then bottom cap and collect the flow-through in a clean tube.
7. Wash the column with 3 resin volumes of Coupling Buffer and combine the flow-through with the above flow-through.
8. Measure the absorbance at 280nm of the combined flow-throughs. After allowing for the dilution of the original sample compare the measurement to the starting material to determine coupling efficiency.
9. Wash the column with 10 resin volumes of Wash Solution.
10. Wash the column with PBS containing 0.05% sodium azide.
11. Replace the bottom cap and add 3-5ml PBS containing 0.05% sodium azide. Seal the column and store upright at 4˚C.

RELATED PRODUCTS

Download our Protein Purification Handbook.



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

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