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A Geno Technology, Inc. (USA) brand name

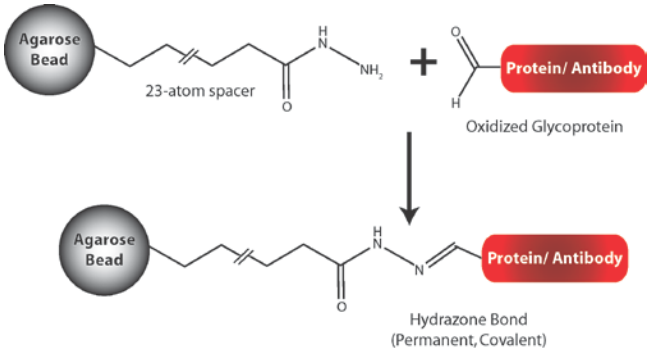
Carbohydrate Immobilization Kit

For Covalent Immobilization of Glycoproteins to Agarose Resin

(Cat. # 786-807)

INTRODUCTION

The Carbohydrate Immobilization kit 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 affinity resin can be reused at least 10 times with no significant loss of activity.
The Carbohydrate Immobilization kit is supplied with carbohydrate coupling resin columns for the preparation of 2ml affinity columns, SpinOUT™ GT-600 Desalting Columns for removing oxidizing reagents, the oxidizing reagent (Sodium *meta*-periodate) and relevant buffers.



ITEM(S) SUPPLIED (Cat. # 786-807)

Part. #	Description	Size
034C-A	Carbohydrate Coupling Resin Columns	5 x 2ml
037C-A	Carbohydrate Coupling Buffer	125ml
224A-A	Aniline	100µl
320S-B	SpinOUT™ GT-600 Desalting Column, 5ml	5
243S-C	Sodium *meta*-periodate (Oxidizing Agent)	25mg
102W-A	Wash Solution	60ml
001J	JAW™ Phosphate Buffered Saline Pack	1

STORAGE CONDITIONS

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

SPECIFICATIONS

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

ADDITIONAL COMPONENTS REQUIRED

- Sodium azide
- 15ml collection/ centrifuge tube

IMPORTANT INFORMATION

- 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 0.1M Sodium Phosphate buffer, pH7.0.
- Gelatin or other carrier proteins win the antibody solution will compete for resin binding sites. Remove these proteins with G-Biosciences' Pearl™ Antibody Clean Up kit (Cat. # 786-803).
- Hydrophobic proteins may require the use of a detergent and additional wash steps to minimize non-specific binding.
- Column flow may be impeded by particulates in the samples. Remove any sediment by centrifugation at 10,000g of filter through a 0.45µm membrane before applying to column.
- Oxidation sensitive proteins, such as metal containing proteins, may require shorter or milder oxidation conditions to prevent functional damage. 1mM sodium *meta*-periodate treatment oxidizes sialic residues, leaving other monosaccharides unmodified. Higher concentrations (10-25mM) oxidize other carbohydrates, such as mannose and galactose.
- Aniline is highly toxic. Wear gloves and handle with extreme care.

PREPARATION BEFORE USE

- PBS with sodium azide: Add the JAW™ Phosphate Buffered Saline (PBS) Pack to 500ml ultrapure water and stir to dissolve. Store buffer at 4°C, for long term storage, sterile filter and supplement with 0.05% sodium azide.
- Equilibrate all components to room temperature before starting.

PROTOCOL

A. Sample Preparation

1. Dissolve 0.5-10mg glycoprotein (i.e. polyclonal antibody) in 1ml Carbohydrate 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, prepare the SpinOUT™ column by removing the top and then bottom caps. Place into a 15ml collection tube.
6. Mark one side of the column and ensure in all centrifugations the mark is facing outwards during centrifugation.
7. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer. This compacts the resin and removes the storage buffer.
8. Add 2.5ml Carbohydrate Coupling Buffer and centrifuge at 1,000g for 2 minutes. Repeat this step once
9. Slowly apply the oxidized glycoprotein to the desalting column and allow the solution to enter the resin bed.
10. Add 0.1ml Carbohydrate Coupling Buffer to the desalting column and allow to enter the resin bed.
11. Centrifuge at 1,000g for 2 minutes to collect the oxidized protein.
12. In a fume hood, prepare 0.2M Carbohydrate Coupling Catalyst. Add 18µl aniline to 1ml Carbohydrate 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. Resuspend the Carbohydrate Coupling Resin in the column by end-over-end mixing and then remove the top then bottom cap. Transfer the column to a 15ml collection tube and centrifuge at 1,000g for 2 minutes. Discard the storage buffer.
2. Equilibrate with 2ml Carbohydrate Coupling Buffer. Centrifuge at 1,000g for 2 minutes and discard flow through. Repeat this step once.
3. Apply the bottom cap to the column and apply the 2-3ml 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.

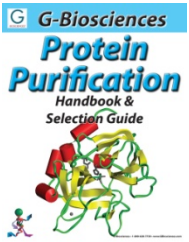
5. After incubation, stand the column upright and allow the resin to settle for 15 minutes.
6. Remove the top then bottom cap and collect the flow-through in a clean tube by centrifuging at 1,000g for 2 minutes. This is the unbound protein. Save this to determine the coupling efficiency.
7. Measure the absorbance at 280nm of the starting protein solution (Step A. 12) and the unbound protein (Step B. 6). After allowing for the dilution of the original sample, compare the measurement to the starting material to determine coupling efficiency.
8. Wash the column with at least 2ml Carbohydrate Coupling Buffer and centrifuging at 1,000g for 2 minutes. Repeat this step three more times.
9. Wash the column with 2ml Wash Buffer and centrifuging at 1,000g for 2 minutes. Repeat this step two more times.
10. Wash the column with 2ml PBS containing 0.05% sodium azide. Repeat this step three more times.
11. Replace the bottom cap and add 3-5ml PBS containing 0.05% sodium azide. Seal the column and store upright at 4°C.

TROUBLESHOOTING

Issue	Possible Cause	Solution
Poor Coupling	Low level of glycosylation on protein.	Find an alternative coupling method.
	Sugars are poorly oxidized.	Ensure oxidation was for 30 minutes.
	Interfering agents in starting material that compete for binding sites.	Ensure sample is dialyzed against a primary amine and sugar free buffer. Avoid Tris.
Low Binding to Affinity Tag	The affinity tag (glycoprotein) may have been damaged during oxidation.	Perform oxidation at 4°C and/or shorten oxidation time.
Loss of binding affinity after multiple uses	Immobilized protein damaged over time by temperature and/or elution conditions.	Generate new column.
	The binding sites and pores are blocked by particulate material in samples.	Centrifuge or filter through a 0.45µm filter before applying to column.
		Use high salt, non-ionic detergents to reduce non-specific binding.
		Increase number of washes.

RELATED PRODUCTS

Download our Protein Purification Handbook.



http://info.gbiosciences.com/complete-protein-purification-handbook

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