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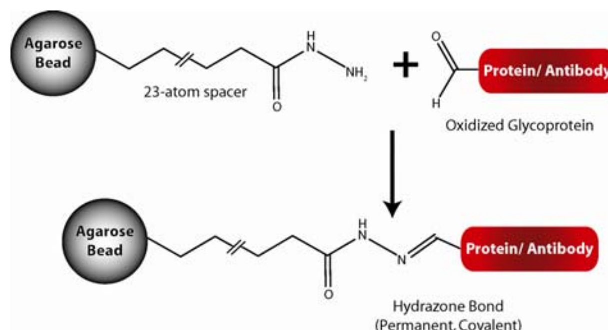
A Geno Technology, Inc. (USA) brand name

Carbohydrate Immobilization Kit

For Covalent Immobilization of Glycoproteins to Agarose Resin

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 generated 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.

KIT COMPONENTS Cat. # 786-807

Part. #	Description	Size
034C-A	Carbohydrate Coupling Resin Columns	5 x 2ml
255O	Optimizer Buffer™ I [5X]	2 x 25ml
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.

IMPORTANT

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

ADDITIONAL COMPONENTS

- Sodium azide
- 15ml collection/ centrifuge tube

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 0.1M Sodium Phosphate buffer, pH7.0.
- Prepare the Optimizer Buffer™ I by adding 10ml 5X Optimizer Buffer™ I to 40ml deionized water. For each column prepare 50ml 1X Optimizer Buffer I.
- 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.

A. SAMPLE PREPARATION

1. Dissolve 0.5-10mg glycoprotein (i.e. polyclonal antibody) in 1ml 1X Optimizer Buffer™ I.
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 Spin-*OUT*™ column by removing the top and then bottom caps. Place into an 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 1X Optimizer Buffer™ I 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 1X Optimizer Buffer™ I 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. Add 1-2ml 1X Optimizer Buffer™ I to the oxidized glycoprotein and continue on to the coupling step. 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 1X Optimizer Buffer™ I. 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 1X Optimizer Buffer™ I 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

I. ***Carbohydrate Coupling Resin*** (Cat. # 786-808) *For coupling glycoproteins, including antibodies to agarose resin.*

II. ***Disposable Columns*** (Cat. # 786-726): *The Spin Columns, 5ml have an internal volume of 8.5ml and is designed for small scale purifications using 15ml centrifuge tubes. Other disposable columns are available.*

III. ***Sodium meta-periodate*** (Cat. # BKC-15) *A mild oxidizing agent that converts carbohydrates to activated active aldehydes.*

IV. ***SpinOUT™ Desalting Columns*** (Cat. # 786-170 to 786-173, 786-703 to 786-708)*: The SpinOUT™ GT-600 and GT-1200 columns are versatile, spin-format columns for the desalting and buffer exchange of protein solutions ranging from 5µl through to 4ml sample volumes. The SpinOUT™ columns are available in two MWCO sizes for >6,000 or >30,000 dalton proteins and are suitable for samples containing as little as 20µg protein/ml.*

For additional related products, visit www.GBiosciences.com.

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