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

A Geno Technology, Inc. (USA) brand name

Thiophilic Adsorption

For the Purification of Immunoglobulins with Thiophilic Resin

(Cat. # 786-266)

INTRODUCTION

Thiophilic adsorption or thiophilic chromatography is a routinely used technique for the low cost, simple purification of immunoglobulins. Thiophilic adsorption was first developed by Porath et al[1] in 1984 and is a group specific, salt-dependent purification technique that has distinct affinity towards immunoglobulins and α_2-macroglobulins. The thiophilic adsorption works on the principle that some proteins in high salt are able to bind to an immobilized ligand that contains a sulfone group in proximity to a thioether group (Figure 1). The bound proteins are then eluted in decreasing salt concentrations.

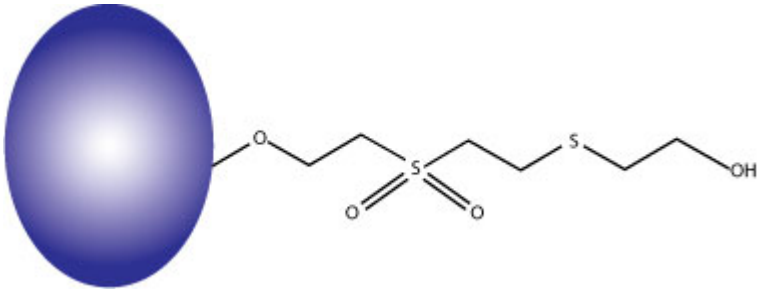

Figure 1: Thiophilic resin structure

G-Biosciences' Thiophilic resin binds immunoglobulins from serum, ascites or tissue culture supernatants and the purified immunoglobulins are then eluted in a near neutral aqueous buffer. G-Biosciences' Thiophilic resin has a high binding capacity (~20mg/ml human IgG/ml resin) and a broad specificity for various species' immunoglobulin molecules.

Thiophilic adsorption has been used to purify other proteins including horseradish peroxidase[2], glutathione peroxidase[3], lactate dehydrogenase[4] and allergens[5].

ITEM(S) SUPPLIED (Cat. # 786-266)

Part. #	Description	Size
067T	Thiophilic Resin Column (3ml resin)	4 columns
041T	TA Equilibration Buffer	1L
040T	TA Elution Buffer	1L
042T	TA Regeneration Buffer	250ml

STORAGE CONDITIONS

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

SPECIFICATIONS

- **Capacity:** >20mg human IgG/ml resin
- **Support:** 6% highly cross-linked agarose

ADDITIONAL MATERIAL REQUIRED

- Anhydrous sodium sulfate (Na_2SO_4) (CAS # 7757-82-6)
- 20% Ethanol

IMPORTANT INFORMATION

- Perform couplings at ~pH8.0, lower pH will result in greater protein binding, however non-immunoglobulin proteins will also bind.

PREPARATION BEFORE USE

Sample Preparation

1. Add 71mg anhydrous sodium sulfate for every 1ml whole serum, ascites or tissue culture supernatant to give a 0.5M final concentration of sodium sulfate.
2. Stir gently to dissolve the sodium sulfate.
3. Centrifuge at 10,000xg for 20 minutes and carefully remove the clarified supernatant.
4. Filter the sample through a0.45µm filter to prevent clogging of the thiophilic resin.
5. Store on ice until ready to use.

PROCEDURE FOR IMMUNOGLOBULIN G PURIFICATION

1. Snap off the bottom tab and place into a 15ml collection tube and allow the storage buffer to drain out.
2. Equilibrate the resin with 4 resin bed volumes of Equilibration Buffer. Discard the flow through.
3. Apply 3-9ml prepared sample to the column and allow to pass through. Save the flow through to monitor the non-bound proteins.
4. Wash the column with 5-10 resin bed volumes of Equilibration Buffer. Monitor flow through at 280nm to determine when all non-bound proteins have been washed from the resin.
5. Elute the bound immunoglobulin with 12 resin bed volumes of Elution Buffer collection the eluent in 3ml fractions. Monitor the immunoglobulin elution by monitoring absorbance at 280nm against water.
6. Regenerate the column by washing with 5 resin bed volumes of elution buffer, followed by 5 resin bed volumes of Regeneration Buffer.
7. Store the column in 20% ethanol at 4°C.

PROCEDURE FOR GENERAL PROTEIN PURIFICATION

Thiophilic resin can purify a variety of proteins. A general protocol is given below; however this protocol should be optimized for the protein of interest. In order to develop successful protein purification a suitable assay for the protein of interest is required.

1. Divide 5ml cellular/tissue lysate containing the protein of interest into 5 equal aliquots.
2. Saturate each aliquot with sodium sulfate to give final concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5M sodium sulfate.
3. Centrifuge the lysates at 10,000xg for 20 minutes to clarify the lysates.
4. Use the cleared lysates in the "Procedure For Immunoglobulin Purification" and compare the eluted protein of interest concentration with that of the initial clarified lysates.
   NOTE: If the protein of interest fails to bind the resin then switch the salt to ammonium sulfate and use higher concentration (≤4M). Repeat the steps outline above.

REFERENCES

1. Porath, J. et al (1984) In Physical Chemistry of Colloids and Macromolecules, Ed. Ranby, B. (Upsala, Sweden), p. 137-142
2. Chaga, G. et al (1992) Biomed. Chromatogr. 6:172-176
3. Huang, K. et al (1994) Biol. Trace Elem. Res. 46:91-102
4. Kminkova, M. & Kucera, J. (1998) Prep. Biochem. Biotechnol. 28:313-317
5. Goubran-Bostros, H. et al (1998) J. Chromatogr. B. Biomed. Sci. Appl. 710:57-65

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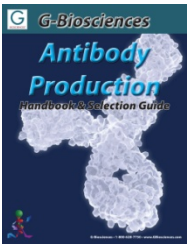

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