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A Geno Technology, Inc. (USA) brand name

Thiophilic Resin

For the Purification of Immunoglobulins

(Cat. # 786-267, 786-268)

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.

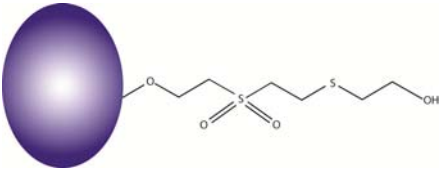

Figure 1: Thiophilic Resin Structure

G-Biosciences' Thiophilic resin binds immunoglobulins, including IgG, IgY and IgM, 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. #	Description	Size
786-267	Thiophilic Resin	10ml resin
786-268	Thiophilic Resin	100ml resin

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 ITEM(S) REQUIRED

- Anhydrous sodium sulfate (Na_2SO_4) (CAS # 7757-82-6)
- Sample Buffer: 20mM sodium phosphate, 0.55M sodium sulfate (pH7.5)
- Equilibration Buffer: 20mM sodium phosphate, 0.5M sodium sulfate (pH 7.5)
- Elution Buffer: 20mM Sodium phosphate, pH7.5
- Regeneration Buffer: 20mM sodium phosphate, 30% propan-1-ol, pH7.5
- Storage Buffer: 20% ethanol
- Empty columns

IMPORTANT

- 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 (1-3ml)

1. For small sample volumes (1-3ml), we recommend a dilution method.
2. Dilute 1ml whole serum, ascites or tissue culture supernatant with 9ml sample buffer
3. Filter the sample through a 0.45µm filter to prevent clogging of the thiophilic resin.
4. Store on ice until ready to use.

Sample Preparation (>3ml)

1. For large sample volumes (>3ml), we recommend a saturation method.
2. Add 355mg anhydrous sodium sulfate for every 5ml whole serum, ascites or tissue culture supernatant to give a 0.5M final concentration of sodium sulfate.
3. Stir gently to dissolve the sodium sulfate.
4. Centrifuge at 10,000xg for 20 minutes and carefully remove the clarified supernatant.
5. Filter the sample through a 0.45µm filter to prevent clogging of the thiophilic resin.
6. Store on ice until ready to use.

PROCEDURE FOR IMMUNOGLOBULIN PURIFICATION

1. Suspend the resin by gently shaking and inverting the resin.
2. Transfer 6ml of the slurry to a 5ml column with a wide bore pipette tip.
3. Snap off the bottom tab and place into a 15ml collection tube and allow the storage buffer to drain out.
4. Equilibrate the resin with 4 resin bed volumes of Equilibration Buffer. Discard the flow through.
5. Apply 3-9ml prepared sample to the column and allow to pass through. Save the flow through to monitor the non-bound proteins.
6. 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.
7. 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.
8. Regenerate the column by washing with 5 resin bed volumes of elution buffer, followed by 5 resin bed volumes of Regeneration Buffer.
9. Store the column in storage buffer at 4°C.
   NOTE: *The reuse of the thiophilic resin is dependent on the nature of the sample. It is highly recommended to only reuse with identical samples to prevent cross-contamination.*

APPENDIX 1: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 a 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.*

APPENDIX 2: IGY PURIFICATION

Additional Item(s) Required

- Anhydrous potassium sulfate (K_2SO_4) (CAS # 7778-80-5)
- Equilibration Buffer: 20mM sodium phosphate, 0.5M potassium sulfate (pH 7.5)
- Elution Buffer: 20mM Sodium phosphate, pH7.5
- Regeneration Buffer: 20mM sodium phosphate, 30% propan-1-ol, pH7.5
- Storage Buffer: 20% ethanol
- Empty columns

Preparation Before Use

The high concentration of lipids in egg yolk will interfere with IgY purification and therefore needed to be precipitated out, using water or PEG. Precipitation by water is described below:

1. Separate the yolks from the egg white.
2. Add 9ml distilled water for every 1ml of egg yolk. Mix and stir slowly at 4°C for at least 6 hours.
3. Centrifuge at 10,000x g at 4°C for 25 minutes to precipitate the lipids. Collect the supernatant containing the IgY.
4. Add 436mg anhydrous potassium sulfate for every 5ml IgY supernatant to give a 0.5M final concentration of potassium sulfate.
5. Stir gently to dissolve the potassium sulfate.
6. Filter the sample through a 0.45µm filter to prevent clogging of the thiophilic resin.
7. Store on ice until ready to use.

Procedure For Immunoglobulin Y (IgY) Purification from Egg Yolk

1. Suspend the resin by gently shaking and inverting the resin.
2. Transfer 6ml of the slurry to a 5ml column with a wide bore pipette tip.
3. Snap off the bottom tab and place into a 15ml collection tube and allow the storage buffer to drain out.
4. Equilibrate the resin with 4 resin bed volumes of Equilibration Buffer. Discard the flow through.
5. Apply the equivalent of ¼ egg yolk prepared sample to the column and allow to pass through. Save the flow through to monitor the non-bound proteins.
6. 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.
7. 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.
   NOTE: *To improve the recovery of total IgY or specific IgY the 0.5M K_2SO_4 in the equilibration buffer can be replaced with 0.6-0.8M Na_2SO_4, however this may affect the purity of the final IgY.*

8. Regenerate the column by washing with 5 resin bed volumes of elution buffer, followed by 5 resin bed volumes of Regeneration Buffer.
9. Store the column in storage buffer at 4°C.
   NOTE: *The reuse of the thiophilic resin is dependent on the nature of the sample. It is highly recommended to only reuse with identical samples to prevent cross-contamination.*

APPENDIX 3: IGM PURIFICATION

Additional Components

- Anhydrous ammonium sulfate ($(NH_4)_2SO_4$) (CAS # 7783-20-2)
- Equilibration Buffer: 20mM sodium phosphate, 0.8M ammonium sulfate (pH 7.5)
- Elution Buffer: 20mM Sodium phosphate, pH7.5
- Regeneration Buffer: 20mM sodium phosphate, 30% propan-1-ol, pH7.5
- Storage Buffer: 20% ethanol
- Empty columns

Preparation Before Use

1. Add 529mg anhydrous ammonium sulfate for every 5ml whole serum, ascites or tissue culture supernatant to give a 0.5M final concentration of ammonium sulfate.
   NOTE: *Add the ammonium sulfate gradually to avoid precipitation of the IgM.*
2. Stir gently to dissolve the ammonium 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 M (IgM) Purification from Whole Serum, Ascites or Tissue Culture Supernatant

1. Suspend the resin by gently shaking and inverting the resin.
2. Transfer 6ml of the slurry to a 5ml column with a wide bore pipette tip.
3. Snap off the bottom tab and place into a 15ml collection tube and allow the storage buffer to drain out.
4. Equilibrate the resin with 4 resin bed volumes of Equilibration Buffer. Discard the flow through.
5. Apply 3-9ml prepared sample to the column and allow to pass through. Save the flow through to monitor the non-bound proteins.
6. 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.
7. 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.
   NOTE: *To improve the recovery of IgM the ammonium sulfate concentration can be increased to 1.0M, however this will result in an increased binding of IgG.*
8. Regenerate the column by washing with 5 resin bed volumes of elution buffer, followed by 5 resin bed volumes of Regeneration Buffer.
9. Store the column in storage buffer at 4°C.
   NOTE: *The reuse of the thiophilic resin is dependent on the nature of the sample. It is highly recommended to only reuse with identical samples to prevent cross-contamination.*

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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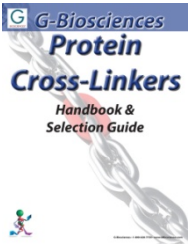

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