Protocol

Purification of Antibodies: Diethylaminoethyl (DEAE) Chromatography

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Because IgG from most species (other than rodents) tends to have an isoelectric point around neutral, two approaches can be used when separating IgG using diethylaminoethyl (DEAE) resins. When serum containing antibodies is applied to DEAE at a slightly acidic pH, the IgG flows through the column while most other serum proteins bind to the DEAE. This method is best performed using a batch method. The DEAE beads can be kept in a disposable syringe containing a polypropylene frit, a glass reactor containing a coarse-sintered glass frit, or other suitable vessel. If the antibody solution is adjusted to a basic pH of 8–8.5, then IgG binds to the DEAE resin. After washing the column, the antibody is eluted by adding a buffer of increasing ionic strength to the column. Prepacked columns of many sizes are available for the isolation of antibodies by DEAE chromatography. Alternatively, DEAE medium can be swelled or prepped according to the manufacturer's instructions and a column can be poured when needed.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Antibody-containing solution (clarified serum, ascites, or an ammonium sulfate or caprylic acid-purified fraction from Protocol: **Ammonium Sulfate Fractionation of Antibodies** [Fishman and Berg 2018a] or Protocol: **Preparation of Antibody Using Caprylic Acid** [Fishman and Berg 2018b])
DEAE agarose or equivalent

This resin can be used multiple times. After completing this protocol, regenerate the DEAE matrix by washing with 0.5 N HCl followed by 0.5 N NaOH.

Reagents for high-pH chromatography (Steps 7–11)

NaCl, for gradient (see Step 10)

Tris HCl (10 mM, pH 8.5)

Reagents for low-pH chromatography (Steps 1–6)

NaCl (1–2 M)

Sodium phosphate buffer (5–7 mM, pH 6.5)

Reagents for SDS-PAGE

Sodium azide (optional; for storage)

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Equipment

Equipment for SDS–PAGE

Glass or plastic column (to perform high-pH DEAE chromatography)

This can be a gravity-fed column or a column with specific fittings for use with pumps (Omnifit, Chemglass). It is important to choose a column of sufficient size to bind the IgG in your sample. A column that contains 2–3 mL of DEAE beads per milliliter of serum (or equivalent) is sufficient for most applications.

pH meter

Stir plate

UV spectrophotometer

Vessel to perform batchwise DEAE binding, washing, and elution (can be a fritted, disposable syringe, coarse-fritted glass vessel, or similar apparatus sufficient for the volume to be used) (to perform low-pH DEAE chromatography)

METHOD

Low-pH DEAE Chromatography

If serum is to be applied to a DEAE column without a prepurification step, dialyze it against 5–7 mM sodium phosphate (pH 6.5) at least three times with a ratio of one part serum to 100 parts dialysate.

1. Wash the DEAE beads according to the manufacturer's instructions. The beads should then be washed with 1–2 M NaCl.
2. Equilibrate the DEAE beads with 20–50 times the volume of the DEAE to be used of low ionic strength (5–7 mM) sodium phosphate buffer (pH 6.5) until the eluate is pH 6.5.
3. Combine the prepared DEAE beads with the antibody solution in a ratio of 2 mL of DEAE beads to 1 mL of antibody solution.
4. Gently agitate the vessel for 1–2 h at room temperature or overnight at 4°C.
5. Isolate the antibody from the supernatant (unbound fraction) by vacuum filtration or another method depending on the vessel used.
6. Determine the antibody concentration using a spectrophotometer at A_{280} and purity by SDS–PAGE, and either further purify the antibody or store appropriately.

For IgG, an A_{280} of 1.35 = 1 mg/mL. If the antibody is to be stored, it can be filter-sterilized, or 0.02% sodium azide can be added. Be sure to document and label that the solution contains sodium azide.

See Troubleshooting.

High-pH DEAE Chromatography

7. Dialyze the antibody sample against 10 mM Tris (pH 8.5). Use at least three changes of the dialysate over 24–48 h; the volume of the dialysate should be at least 100× the volume of the antibody solution.
8. Wash the column with 10–20 column volumes of 10 mM Tris-HCl (pH 8.5). If the column material has been regenerated using HCl/NaOH with or without high salt, the pH of the eluate should be monitored and the washing deemed complete when the pH is 8.5.
9. Apply the antibody solution to the column. Wash the column with at least 10 column volumes of 10 mM Tris (pH 8.5). The eluent can be monitored for protein content; when no protein is observed and the pH is stable, the washing is considered complete.
10. Elute IgG from the washed column using the same buffer as above (10 mM Tris at pH 8.5) containing a gradient of 50–250 mM NaCl. The gradient volume should be between 10 and 20 times the volume of the DEAE used. Collect 20–30 appropriate-sized fractions.

11. Determine the protein concentration in each fraction using a spectrophotometer at A_{280} , determine the antibody purity by SDS–PAGE, and if needed, either further purify the antibody or store appropriately.

For IgG, an A_{280} of 1.35 = 1 mg/mL. If the antibody is to be stored, it can be filter-sterilized or 0.02% sodium azide can be added. Be sure to document and label that the solution contains sodium azide.

See Troubleshooting.

TROUBLESHOOTING

Problem (Step 6, Step 11): Antibody recovery is lower than expected.

Solution: Should the recovery of the antibody be lower than expected, repeat the process. The pH can be raised or lowered slightly (0.5 pH units at a time) to attempt to improve recoveries because IgGs from different species can have slightly different pIs.

REFERENCES

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Cold Spring Harb Protoc doi: 10.1101/pdb.prot099119.

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