Protocol

Purification of Labeled Antibodies Using Size-Exclusion Chromatography

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Many antibody labeling procedures call for a desalting or purification step requiring size-exclusion chromatography (SEC). The method outlined here contains information needed to desalt an antibody conjugate. Similar procedures would be used for ion-exchange chromatography using a gradient of increasing ionic strength. Resins can be purchased in bulk (as in this protocol), or commercially available columns are available.

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

DiH₂O

Labeled antibody solution

Running buffer: PBS, Tris, HEPES, MES (type and pH will depend on the application)

Size-exclusion gel matrix (e.g., Sephadex; choice dependent on size of conjugate; see Table 1)

Table 1 provides a list of SEC Sephadex resins and the effective separation range of each. Typically manufacturers give a volume/milliliter value. Your volume of sample should be no more than 5% of the column volume for desalting and no more than 2% of the column volume when separating molecules that are close in size.

Equipment

Chromatography equipment (optional)

Prepoured columns are available, but even common laboratory materials (i.e., 10-mL syringe with filter paper or frit) can be adapted for use.

Glass or polypropylene column

Glass or polypropylene tubes

Glassware (beaker or flask)

Peristaltic pump (optional)

Spectrophotometer

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TABLE 1. SEC Sephadex resins and the effective separation range of each

Sephadex	MW range	Application
G-10	≤700	Very-low MW substances; desalting; buffer exchange; peptides
G-15	≤1500	Very-low MW substances; desalting; buffer exchange; peptides
G-25	1000–5000	Most common for desalting; buffer exchange; small peptides and proteins
G-50	1500–30,000	Desalting; buffer exchange; small peptides and proteins
G-75	30,000–80,000	Protein separation
G-100	4000–150,000	Protein conjugate separation
G-150	5000–300,000	Protein conjugate separation; protein complexes—low noncovalent interactions
G-200	5000–600,000	Protein conjugate separation; protein complexes—low noncovalent interactions

METHOD

1. Swell the resin in 200–300 mL of DiH₂O overnight at room temperature.
Some resins require different swelling conditions including degassing. Refer to the manufacturer's instructions.
2. Decant the liquid without losing the swelled gel.
3. Wash the gel twice with DiH₂O, letting the matrix settle and decanting the liquid.
4. Replace the DiH₂O with running buffer. Let the gel settle, decant, and add fresh running buffer. Repeat this again.
5. Pour the swelled gel into a column and allow it to settle using either gravity or a pump.
6. When adding additional matrix, gently stir the interface with a sterile pipette.
Keeping the poured resin as continuous as possible will ensure the best separation.
7. Equilibrate the column with either 10 column volumes of buffer, or if the buffer was replaced during the swelling process (Step 4), then two column volumes will be sufficient.
8. Expose the gel bed and gently load the sample onto the gel bed, minimizing disturbance of the surface.
9. Allow the sample to completely enter the gel bed, and then begin to gently add running buffer to the gel bed.
10. Collect 0.5-mL fractions from the column.
11. After several milliliters of running buffer have entered the gel bed, additional buffer can be added to the top of the gel.
12. Measure the OD of the fractions in a spectrophotometer. The first peak is the antibody and should begin to elute in approximately the sixth fraction. Pool the main peak and measure the OD again to determine the antibody concentration.
See Troubleshooting.
13. Store the antibody conjugate as described above and in Introduction: Antibody Purification and Storage (Berg and Fishman 2019).

TROUBLESHOOTING

Problem (Step 12): The protein elutes too quickly (crack in column).

Solution: Collect the sample, concentrate, repour the column, and repeat the protocol.

Problem (Step 12): The protein elutes too slowly (wrong sizing matrix chosen).

Solution: Use a different matrix that separates in a smaller MW range.

RELATED INFORMATION

A general review of chromatography theory and techniques can be found in Snyder et al. (2010).

REFERENCES

Berg EA, Fishman JB. 2019. Antibody purification and storage. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top099101.

Snyder LR, Kirkland JJ, Dolan JW. 2010. *Introduction to modern liquid chromatography*, 3rd ed. Wiley, New York.

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