NIH AIDS Research & Reference Reagent Program
20301 Century Boulevard
Bldg. 6, Suite 200
Germantown, MD 20874
USA

Phone: 240 686-4740
Fax: 301-515-4015
www.aidsreagent.org

Column Purification of Hybridoma 183 (HIV-1 p24) Supernatant

Dr. Bruce Chesebro and Dr. Kathy Wehrly

The following protocol describes the purification of an anti-p24 Gag monoclonal antibody produced by Hybridoma 183 (Clone H12-5C). Two to three liters of culture supernatant can be purified in a single run to yield approximately 10-15 µg of purified antibody per ml of starting material. The purified IgG can be used to coat ELISA plates for development of an antigen capture assay.

Reagents:

Hybridoma 183	Clone H12-5C (Repository Catalog #1513)
Hybridoma Supernatant	2-3 liters, prepared from Hybridoma 183 (smaller volumes can be used if desired)
Affinity Purification Column	3.0 ml column (10 mm x ~38 mm) containing rec-Protein G Sepharose 4B (Zymed, Catalog #10-1242). One ml of gel is capable of binding 22 mg human IgG.
PBS	10 mM Na_2HPO_4 (dibasic), 150 mM NaCl, pH 7.4; prepare approximately 5 liters
Elution Buffer	0.2 M glycine HCl, pH 2.5; prepare approximately 0.5 liters
Neutralizing Buffer	1.0 M Na_2HPO_4, dibasic

Procedure:

1. Grow Hybridoma 183 as described in the data sheet included with the cells, and pool the collected tissue culture supernatant. Store the supernatant at 4°C until purification is performed.

2. Prepare the affinity purification column and store it at 4°C. The following two steps must be performed in a cold room at 4°C.

3. Equilibrate the column with 200 ml of PBS (chilled to 4°C) and monitor the flow through to bring the A_{280} to baseline.

4. Apply the hybridoma supernatant (chilled to 4°C) to the column at a flow rate of 0.5-2 ml/min.

5. Once the run has been completed, move the column from the cold room to a room temperature area and warm it by washing with 200 ml room temperature PBS. Collect the fractions periodically and monitor the A_{280} to determine when washing is complete.

6. Elute the bound antibody with the elution buffer. Collect fractions of 3-5 ml into tubes that contain neutralizing buffer, monitoring the A_{280} periodically. The ratio of eluate to neutralizing buffer should be made 4:1, and the pH of the fractions upon mixing should be 7-7.5. Some monoclonal antibodies require a lower pH elution buffer; if this procedure is used with other monoclonals, it may be necessary to lower the pH of the elution buffer to 2.0, and repeat this step.

7. Pool the fractions with the peak absorbance readings.

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8. Using dialysis tubing with a molecular weight cut-off of 10,000-20,000, dialyze the eluted antibody against 2 liters of cold PBS for 2 hours at 4°C.

9. Replace the PBS with 2 fresh liters of cold PBS and dialyze overnight at 4°C.

10. Carefully remove the purified antibody from the dialysis tubing and measure the volume.

11. Using the dialysis buffer as blank, measure A_{280} and calculate the antibody concentration as follows:

    A280/ over {1.47 x dilution factor)} = mg/ml

    mg/ml x total ml lysate = total mg antibody

12. If necessary, concentrate the preparation using a Centricon 10 or Amicon system.

13. The purified antibody can be stored at 4°C if the concentration is 0.5-3.0 mg/ml and the preparation is sterile or preserved with azide. Otherwise, aliquot the antibody and store at -70°C. Avoid repeated freeze-thaws.

14. Purified 183 antibody can be used directly, biotinylated, or conjugated by other means for use in an antigen capture ELISA. Optimal coating concentrations may vary between different antibody lots, and should be determined separately for each batch.