Fibronectin Purification

Materials

Gelatin Sepharose 4B gelatin (GE Healthcare/Amersham catalog number 17-0956-01) in column
    Can set up with a pump instead of pouring in volumes with a graduated cylinder
Bovine Plasma (Pel-Freez Catalog number 37140-1) (Choose EDTA or Citrate solution)
Ring stand and clamp
Discard beakers
500 ml High pH Regeneration buffer
500 ml Low pH Regeneration buffer
Stock solutions PMSF in 100% Ethanol (4C)
2 L Wash Buffer (1)
200 ml Wash Buffer (2) - can be made from Wash Buffer (1)
1 L 4x Dialysis Buffer
    +3 L MilliQ Water at 4C
100 ml Elution Buffer - Made from 25 ml 4x Dialysis buffer
Dialysis Tubing - 25 MWCO large diameter tubing (Spectra catalog number)

Day 1:

Remove Bovine Plasma (500 ml Pel-Freez cat number 37140-1) from -80C and allow to thaw. Store at 4C overnight.

Prepare Buffers (Day 2 can also be used for buffer preparation, See Buffer Sheet for Details)
    Wash Buffer (1): 1 L 1xPBS, 1 mM PMSF (add right before use), 2 mM EDTA pH 8.0
    Wash Buffer (2): 200 ml 1PBS, 1 mM PMSF (add right before use), 2 mM EDTA pH 8.0,
        1 M NaCl
    Dialysis Buffer: 4 L 10 mM Caps, 0.15 M NaCl, 2 mM EDTA pH 8.0,
        1 mM PMSF (add right before use), pH 11.0 Keep at 4C
    Elution Buffer: 100 ml Dialysis Buffer, 4 M Urea, pH 11.0

Prepare Regenerating buffers (Listed below, see Buffers Sheet for details)
    High pH: 500 ml 0.1 M Trisma Base, 0.5 M NaCl, pH 8.5
    Low pH: 500 ml 0.1 M Sodium Acetate, 0.5 M NaCl, pH 4.5
    Wash Buffer (1): 1 L 1x PBS, 1 mM PMSF (add right before use), 2 mM EDTA pH 8.0.

Regenerate column by alternating 3x between the High and Low pH buffers, 150 ml at a time. Begin with High. On the third, and last wash by buffer, use 200 ml. Do not let gelatin bed dry.

Wash the gelatin bed with Wash Buffer (1), run full 1 L through the column. Do not let gelatin bed dry. If there are bubbles or irregularities in the bed, gently stir with a serological pipette while at this step.

Replace column stoppers and store overnight at 4C. The equivalent of at least one gelatin bed of Wash Buffer (1) should remain in the column, above the bed, when storing.

Day 2:

Removed Bovine Plasma from 4C. Add PMSF solution to plasma so that final PMSF concentration is 1 mM. Mix gently and transfer to centrifuge tubes. Centrifuge at RT for 30 minutes at 13,700 xg. Transfer supernatant to a graduated cylinder and discard pellet.

Remove gelatin column from 4C. Unstopper column and gently add centrifuged plasma to the column. Be careful not to disturb the gelatin bed. Do not allow the gelatin to dry. Run full volume of Plasma.

Wash Column with 600 ml of Wash Buffer (1). Be careful to not disturb the column bed and not to allow the bed to become true. This is true throughout the washing steps, and especially during elution.

Wash column with 200 ml of Wash Buffer (2)

Wash column with 100 ml of Wash Buffer (1) - Important, save last 100 ml of Wash Buffer (1) to clean the gelatin bed after elution.

Elute with 50 ml of elution buffer. Begin collection with a 50 ml falcon tube on ice. It may take several drips before elution occurs. The first few drops after elution are the most concentrated. Pool entire elution volume into one, fraction collection is not necessary. It is very important to keep everything at 4C from this point on. Always keep fibronectin elution on ice or in the cold storage room.

Dialyze elution overnight at 4C in 4 L of dialysis buffer. Use Spectra 25 MWCO large dialysis tubing (catalog number)

CLEAN AND STORE COLUMN.

Clean the gelatin bed with remaining 100 ml of Wash Buffer (1). Run 50 ml of 30% Ethanol through the gelatin bed for additional cleaning. Store the column in 50 ml of 30% ethanol at 4C.

Day 3:

Collect dialyzed fibronectin into a 50 ml tube. Combine and measure OD280 (nanodrop). May need to dilute small sample 1:1 with dialysis buffer. Dilute fibronectin until a concentration of 1 mg/ml with dialysis buffer.

Make 1 ml aliquots of the 1 mg/ml fibronectin. Flash freeze and store at -80C.