Preparation of Cellular Fibronectin

Materials

1. Medium: DME with glutamine, 10% fetal calf serum, penicilin and streptomycin.

2. Hanks balanced salt solution with 10 mM HEPES, pH 7.4 (use 1 M HEPES for preparation), 37^{o}C, 20 ml per 100 mm dish or 50 ml per large TC flask (175 cm^{2}).

3. 2 mM PMSF, 4 mM glutamine in DME (no serum), 37^{o}C, 10 ml per 100 mm dish or 25 ml per TC flask.

4. 1 M urea, 2 mM PMSF in DME, 5 ml per 100 mm dish or 12 ml per TC flask.

5. Shaker in a 37^{o} room or incubator.

6. SS34 rotor, prechilled in a Sorvall centrifuge.

7. 0.2 M CAPS, pH 11.

8. 0.15 M NaCl, 1 mM $CaCl_2$, 10 mM CAPS, pH 11, need 4000 ml.

9. 5 N NaOH.

10. Ultrapure ammonium sulfate.

Note: Always use polyethylene or polypropylene containers (not glass) and polyethylene transfer pipets when dealing with fibronectin.

Procedure

1. Culture 7 day chick embryonic heart fibroblasts on two 100 mm culture dishes.

2. Change medium 6-12 hrs after primary plating.

3. Allow cells to grow 3-4 days without medium change to select for fibroblasts.

4. Trypsinize primary cultures: rinse with STE, then apply 0.05% Trypsin.

5. Plate cells at 6-8 x 10^{6} cells per 100 mm dish or 1.3-1.8 x 10^{7} cells per large TC flask (175 cm^{2}).

6. Allow cells to reach confluency.

7. Rinse confluent cultures 4x with Hanks balanced salt solution, 10 mM HEPES (buffer 2).

8. Rinse with 2 mM PMSF, 4 mM glutamine in DME (buffer 3), 37^{o}C. Incubate 1 hr at 37^{o}C on a shaker, shake at one revolution per minute.

9. Remove solution and rinse with the same solution. Remove all liquid.

10. Add DME containing 1 M urea and 2 mM PMSF. Incubate and shake for 2 hr at 37^{o}C as in step 8. Use 10 ml for each TC flask or 4.5 ml for each 100 mm dish.

11. Collect medium into 50 ml conical tubes or plastic SS34 tubes. Spin at 14,500 rpm for 15 min in a SS34 rotor at room temperature.

12. Pool the supernatant, measure volume. Precipitate with 70% ammonium sulfate (0.472 g/ml).

13. Allow to stand for 1-2 hrs at 4^{o}C.

14. Spin at 14,500 rpm, 4^{o}C for 15 min in a SS34 rotor.

15. Resuspend pellets in 0.2 M CAPS pH 11.0. Use 1 ml / 20 ml urea extract solution measured in step 12. Mix with a polyethylene transfer pipet and immediately adjust pH to 11.0 with 5 N NaOH. Clean up electrode afterwards.

16. Dialyze against 1000 ml buffer 8 for 16-18 hrs at 4^{o}. Stir vigorously and cover flask well to prevent CO_2 from entering.

17. Reprecipitate with 50% ammonium sulfate (0.313 g/ml).

18. Centrifuge in a SS34 rotor at 14,500 rpm, 4^{o}C for 15 min. Resuspend pellet in a small volume of buffer 8 and dialyze for 24 hr against 1000 ml buffer 10 at 4^{o}C, change buffer 2 times.

19. Centrifuge as above (or in an ultracentrifuge), pool supernatant.

20. Expect ~0.1 mg per 175 cm^2 TC flask. Store in liquid nitrogen.
