Endothelial Cell Growth Supplement

Materials:

- Bovine Hypothalamus
- NaCl solution (NaCl (Merck 1.06400), 8.77g/l in H_2O, pH 7.0)
- Streptomycin Sulphate solution (Streptomycin Sulphate (Merck 1.10117), 15% in H_2O)
- Ammonium Sulphate (Merck 1.01217)
- 0.01M Sodium Phosphate buffer ($NaH_2PO_4.H_2O$ (Merck 1.06346), 0.386g/l, Na_2HPO_4 (Merck 1.06586), 1.022g/l in H_2O, pH 7.2)
- Mull compressor
- Cellulose dialysis tube (MWCO 2000, Spectra/Por® 6, Order no: 132633)
- 0.45µm and 0.22µm sterile-filters
- general laboratory equipment (Beakers, Funnels, Measuring cylinders, Magnetic stirrer, …)

Procedure:

1. Add 600g Hypothalamus to 1000ml cold (4°C) NaCl solution
2. Homogenize for 30 minutes at 4°C
3. Extract supernatant with mull compressor
4. Centrifuge for 60 minutes at 8000rpm, 4°C
5. Filter supernatant with mull compressor into a measuring cylinder, measure total recovered supernatant volume (VOL1)
6. Add 1/20 VOL1 Streptomycin Sulphate solution drop wise in all directions
7. Stir at 4°C overnight
8. Centrifuge for 60 minutes at 11000rpm, 4°C
9. Collect supernatant, measure volume
10. Add 290g/l Ammonium Sulphate (slowly, 2 spoons every 5 minutes) to supernatant at 4°C to reach a final concentration of 50%
11. Stir at 4°C overnight
12. Centrifuge for 30 minutes at 13000rpm, 4°C
13. Collect supernatant, measure volume
14. Add 250g/l Ammonium Sulphate (slowly, 2 spoons every 5 minutes) to supernatant at 4°C to reach a final concentration of 90%
15. Stir at 4°C overnight
16. Centrifuge for 30 minutes at 13000rpm, 4°C
17. Collect the pellet, redissolve in distilled H_2O (50-100ml, until pellet is completely dissolved)
18. Dialyse against 5l 0.01M Sodium Phosphate buffer for 48 hours (change buffer every 12 hours)
19. Sterile filter first with 0.45µm filter, then with 0.22µm filter
20. Aliquot and store at -80°C

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