PC12 Cell Culture and Fusion

Cell Culture

Materials

1. Falcon Primaria culture dishes.

2. Culture medium: DME (or F12K) with glutamine, supplemented with 7% heat-deactivated horse serum (56oC for 30 min), 7% fetal calf serum, 50 units penicillin, 50 ug/ml streptomycin, 37^{o}C.

3. ECL matrix (E-C-L cell attachment matrix, Upstate Biotech, 08-110). Stored as 250 ul aliquots in 15 ml conical tubes at -80^{o}C. Keep it cold all the time otherwise it will form a gel.

4. F12K medium (no additives), for diluting ECL matrix.

5. Coverslip dishes. Add 12 ml of cold (4oC) F12K medium to a tube of ECL aliquot, vortex. Pipet 2-3 ml on each coverslip dish and incubate for at least 1 hr in the incubator. Replace the ECL solution with culture medium before use.

Procedure

1. Seed cells on Primaria dishes. Cells grow in clusters of 3-5. Feed 3 times weekly and exchange only 2/3 of the medium.

2. When the density is high or clusters get large (more than 6-8 cells per cluster), the cells should be dispersed by gentle pepeting, without the use of STE or trypsin. Disloged cells are replated on fresh dishes at a lower density (1:3 or 1:4). Do not dilute too much.

3. DME-based medium may be replaced by F12K-based medium 2-7 days before fluorescence observation. This reduces autofluorescence.

4. Store cells by suspending in 90% medium, 10% DMSO, freezing slowly as for other cell lines before transfering to liquid nitrogen.

Reference

L.A.Greene, J.M.Aletta, A.Rukenstein and S.H.Green (1987) PC12 pheochromocytoma cells: culture, nerve growth factor treatment, and experimental exploitation. *Methods Enzymol.* 147:207-216.

Fusion

Materials

1. PC12 cell stock in 60 mm dishes. The optimal density is clusters of 3-5 cells spread over the dish.

2. PEG/DME: autoclave 10 g PEG (PEG1500, BDH limited, 29575) in a 100 ml medium bottle, then add immediately 10 ml warm (37^{o}C) DME (no additive) and mix by swirling until the solution is homogeneous. Warm up to 37^{o} before use. Store at room temperature and warm up to 37^{o}C before use.

3. DME (no additive), 37^{o}C.

4. F12K culture medium, 37^{o}C (see Cell Culture).

5. STE, 37^{o}.

6. Trypsin, 0.25%, 37^{o}.

Procedure

1. Remove medium, gently place 2 ml of PEG/DME on the dish.

2. Incubate for exactly 1 min.

3. Remove PEG/DME and gently rinse the dish with 2 ml DME, 3 times.

4. Add fresh F12K culture medium, incubate for 20 min at 37^{o}C.

5. Remove medium and rinse gently with STE.

6. Gently rinse with trypsin and remove immediately.

7. After 1-2 min, pipet cells off the dish and seed onto fresh dishes (or coated coverslip dishes) containing the culture medium. NGF is usually included in the medium to start priming (see Induction of Differentiation).

Reference

P.H.O'Lague and S.L.Huttner (1980) Physiological and morphological studies of rat pheochromocytoma cells (PC12) chemically fused and grown in culture. *Proc. Natl. Acad. Sci.* USA 77:1701-1705.

Induction of Differentiation

Materials

1. Nerve growth factor (2.5S NGF, Bioproducts for Science). Prepare 1 mg/ml stock in 50 mM NaAc, pH 5.0. Sterile filter and store as 5 ul aliquots in 15 ml conical tubes at -80^{o}C.

2. NGF medium: Add 1 ml of F12K medium to each tube of NGF aliquot and vortex. Then take 120 ul and add to 3 ml of medium to obtain a final concentration of 200 ng/ml.

3. Forskolin (Calbiochem 344270) or 7-beta-deacetyl-7 beta-(gamma-N-methyl-piperazino)-butryl forskolin (Calbiochem 344273). Prepare 20 mM stock in 95% ethanol and store at -20^{o}C.

4. Forskolin-NGF medium: NGF medium with 7.5 ul forskolin stock.

Procedure

1. Culture cells in NGF medium for 2-3 days.

2. Remove medium and add forskolin-NGF medium. Rapid neurite extension should be apparent within 20 min.
