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SH-SY5Y culturing

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We use this protocol and it's working

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Abstract

SH-SY5Y neuroblastoma cells were cultured in a 1:1 mixture of Ham's F-12 and DMEM growth medium supplemented with 10% FBS, non-essential amino acids (NEAA: 0.1 mM of: glycine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline and L-serine) and penicillin/streptomycin antibiotic cocktail (50ng/ml) at 37°C and 5% CO_2. Cells were passaged at around 80% confluency using trypsin digestion. Culture medium was removed from cells, centrifuged at 1000 RPM for 5 minutes to remove floating cells and stored at 80°C for analysis. Cells were washed once in PBS and trypsin was added (0.25% trypsin in Versene). Following an incubation of 2 minutes, the plates were tapped to detach cells. The trypsin was neutralised in 9 volumes of normal growth medium. For continued culturing, a fraction of cells was transferred to a falcon tube and pelleted for 10 minutes at 1000 RPM (200 x g). Pellets were resuspended in the appropriate volume of normal medium and split into new plates. If required, cells were counted prior to seeding using the C-Chip NanoEnTek DHC-N01 counter. Cell line stocks were generated by resuspending cell pellets in 1 mL of freezing medium (10% DMSO in FBS).

Safety warnings

Biological Hazards:

·The culturing of human cells is a class II activity and all appropriate safety issues need to be addressed.

·If using GMOs read the Laboratory Codes of practice for working with GMOs

Characteristics

1
- ATCC # CRL-2266
- Human neuroblastoma cell line, cloned from SH-SY5, which is from SH-SY, which is from SK-N-SH. The original cell line was isolated from a woman's metastatic bone tumor in 1970.
- Known to be dopamine beta hydroxylase active, acetylcholinergic, glutamatergic and adenosinergic.
- Grown as adherent cells; dividing cells can form clusters of cells which are reminders of their cancerous nature, but certain treatments such as retinoic acid and BDNF can force the cells to dendrify and differentiate.
- Doubling time = 48h

Complete growth medium

2
- 1:1 mixture of

- F12 (LT # 31765-068, 1802 mg/L glucose and 110 mg/ml sodium pyruvate) and,
- DMEM (# 61965-059; 4500 mg/L and no pyruvate) both Glutamax medium

Or DMEM/F12 (#31331028)

- Fetal bovine serum (FBS, 10% final) – 1 / 10 dilution
- Pen / Strep (LT # 15140-122, 50 U/ml and 50 ng/ml final) -- 1 / 200 dilution
- MEM / NEAA (LT # 11140-035) -- 1 / 100 dilution
- As required -- Sodium pyruvate (Sigma # S8636, 1 mM final) – 1 / 100 dilution
- As required -- Uridine (Sigma # U3003, stock of 25 mg/ml in H2O filter-sterilised; 50 ug/ml final) – 1 / 500 dilution

- Omit uridine and pyruvate to select against ρ^0 cells (selective medium)
- uridine not necessary for general cell growth but helps MRC defective cells

- As required, as permitted by DSO -- Fungizone (LT # 15290-026; original of 250 ug/ml) – 1 / 100 dilution

Subculturing by trypsinisation, and cell counting

3
1. Wash cells grown onto 10 cm Ø plate with PBS.
2. Add 1 ml diluted trypsin (4 ml of 2.5%, LT # 15090-046, into 100 ml Versene LT # 15040-033) and leave at R/T for several mins.
3. Tap the plates and add 2 ml complete medium to stop trypsination.Pipette trypsinised cells to break down clumps of cells, triturate using 21G needles if necessary.
4. To a 50 ul sample, add equal vol of 0.4% Trypan blue (Sigma # T8154 or LT # 15250-061); fed it to a hemacytometer.
5. Optional -- to pellet cells and resuspend into fresh medium.
6. Replate the cells accordingly; typically 8 ml medium is used for 10 cm Ø plate.
7. Subcultivation ratio of 1:4 to 1:16 is recommended; medium renewal every 2-3d.

Freezing and thawing

4 1.After centrifugation to pellet cells, resuspend in Freezing medium (90% FBS / 10% DMSO filter sterilised) and aliquot into Freezing vials; typically a confluent 10 cm Ø plate of ~ 6 million cells in 1ml.Freeze cells in a stepwise fashion from -80'C to liq N.
2.Thaw cells at 37'C.