SH-SY5Y CELL CRYOSTOCKS

MATERIALS

Complete Medium
Trypsin 0.25% (Sigma T4049-100ML)
Cryostock Tube

NOTES

1. Always use sleeve protectors in the hood.
2. There is anecdotal and experimental evidence that through continuous subculture (or passaging) SH-SY5Y cells start to lose their neuronal characteristics and the potential to generate neurites. The consensus is that cells should be maintained below passage (P) 20.
3. Split cells or cryopreserve when they are ~ 75% confluent (in logarithmic growth).

PROCEDURE

1. Complete media

500 mL	ATCC-formulated Eagle's Minimum Essential Medium
500 mL	F12 Ham's Medium
100 mL	Fetal Bovine Serum
10 mL	Nonessential amino acids
10 mL	Pen-Strep
1 mL	L-glutamine

2. Freezing solution
   9.5 mL Complete medium
   0.5 mL DMSO
3. Warm complete media solution and trypsin solution to 37 ºC in incubator until warm to touch.
4. Carefully remove spent media leaving cells adhered to the flask, and add to a 50 mL conical tube.
5. Add 5 mL trypsin solution to culture flask and gently distribute over surface of cells. Let stand 5 minutes.
6. Check cells under the microscope to ensure cells have detached. If necessary, loosen cells by gently rapping on the side of the culture flask.
7. Transfer cells in trypsin solution to the 50 mL centrifuge tube with suspended cells.
8. Pellet cells by centrifugation at 1500 rpm for 5 minutes.
9. Decant media and trypsin solution.
10. Resuspend cells in 10 mL freezing solution by gently mixing.
11. Aliquot 1 mL of suspended cells into 2.0 mL cryostock tubes prelabeled with the cell line, date and your initials.
12. Place the aliquots into the Mr. Freeze freezing apparatus in -80ºC and incubate overnight.
13. The next day, move to liquid nitrogen tank for long term storage at a temperature below 130°C, preferably in liquid nitrogen vapor.

SH-SY5Y FROM CRYOSTOCK

MATERIALS

- Hams F12 media
- Fetal Bovine Serum
- Pen-Strep Solution
- Cryostock culture
- Cell Culture Flask
- ATCC-EMEM
- NEAA
- L-glutamine
- Pen-strep

NOTES

1. Always use sleeve protectors in the hood.
2. There is anecdotal and experimental evidence that through continuous subculture (or passaging) SH-SY5Y cells start to lose their neuronal characteristics and the potential to generate neurites. The consensus is that cells should be maintained below passage (P) 20.
3. Chemicals such as retinoic acid can induce differentiation in SH-SY5Y cells. Under these conditions the cells express cell neurites and adopt a neuron-like phenotype.
4. Neuronal differentiation, however, should be performed using a chemical agent such as retinoic acid in an appropriate basal medium (such as Neurobasal Medium +B27 Supplement).
5. Standardized stocks of SH-SY5Y cells have the advantage of the proliferative potential of an immortal cancer cell line combined with ability to be differentiated into neuron-like cells that can then be used in functional assays.

PROCEDURE

1. Complete media

500 mL	ATCC-formulated Eagle's Minimum Essential Medium
500 mL	F12 Ham's Medium
100 mL	Fetal Bovine Serum
10 mL	NEAA
10 mL	Pen-Strep
1 mL	2 mM L-glutamine

2. Warm complete media solution to 37 ºC in the incubator to allow the medium to reach its normal pH.
   a. It is important to avoid excessive alkalinity of the medium during recovery of the cells.
3. Label the flask with the culture, date, and generation of splitting.
4. Pipette 20 ml of complete media solution into 75cm^2 cell culture flask(s).
5. Thaw the cryostock tube quickly by mixing with warmed media. When the culture is thawed, pipette it into the flask(s). Gently mix the solution.
6. Incubate the culture at 37ºC in the CO_2 incubator for at least 4 hours or overnight.

7. Place a 20 ml aliquot of complete media in the incubator.
8. When the new media is warmed, decant off the old media, and transfer the 20 ml of new media into the culture.
    a. To save SH-SY5Y cells in suspension, centrifuge down, resuspend in warmed media and add back to the culture flask.
9. Exchange media as necessary (~every 3-4 days).

SH-SY5Y CELL SPLITTING/PLATING

MATERIALS

EMEM:F12 complete media
Fetal Bovine Serum
Pen-Strep Solution
Cryostock culture
Cell Culture Flask
Poly-l-lysine

PROCEDURE

1. In the hood, sterile filter 1 mL poly-l-lysine stock into 50 mL ultrapure water. Add 100 uL per well to your 96-well plates and let sit at least 1 hour.
2. Aspirate off the poly-l-lysine solution, leave open in the hood and wait to plate until poly-l-lysine is completely dry.
3. Complete media

500 mL	ATCC-formulated Eagle's Minimum Essential Medium
500 mL	F12 Ham's Medium
100 mL	Fetal Bovine Serum
10 mL	NEAA
10 mL	Pen-Strep
1 mL	2 mM L-glutamine

4. Pre-warm complete media and trypsin solution to 37 ^{o}C in the incubator.
5. Carefully remove suspended cells and spent media into a 50 mL conical tube leaving cells adhered to the flask.
6. Add 5 mL trypsin solution to culture flask and gently distribute over surface of cells. Let stand 5 minutes.
7. Check cells under the microscope to ensure cells have detached. If necessary, loosen cells by gently rapping on the side of the culture flask.
8. Transfer cells in trypsin solution to the 50 mL centrifuge tube with suspended cells.
9. Pellet cells by centrifugation at 1500 rpm for 5 minutes.
10. Decant media and trypsin solution.
11. Resuspend cells in 5 mL fresh media by gently mixing.
12. In a new tissue culture flask, combine 20 mL fresh media and with desired concentration of cell suspension.
    a. 1 mL = ~5 days
    b. 0.5 mL = ~7 days
    c. 0.25 mL = ~10 days
    *Note: media needs exchanged every 3-4 days.
13. Label the flask with the culture, date, and generation of splitting.
14. Return the culture to the incubator.
15. Add 50 uL cell solution to 450 uL media.
16. Add 10 uL cell solution to the hemocytometer and count the cells in all four quadrants. Divide by 40.

a. Plate at 1 million cells/mL in 96 well plate. (100 uL per well)
b. Plate at 50,000 cells/mL on coverslips in 24 well plate (1 mL per well)