Differentiation of SK-N-SH Cells with *all –trans–* Retinoic Acid (RA) Prior to Harvesting

ENCODE4 - Version 1

Materials List

1. MEM (CORNING Cellgro; Cat # 10-10-CV or 10-10-CM)
2. Heat Inactivated Fetal Bovine Serum (CORNING; Cat # 35-016-CV)
3. Penicillin-Streptomycin 10,000U/ml (Life Technologies, Cat # 15140 or Corning Cellgro, Cat # 300-002CI)
4. Phosphate Buffered Saline (1X PBS) w/o Ca^{2+}, Mg^{2+} (CORNING Cellgro; Cat # 21-040-CM)
5. TrypLE Express (Life Technologies; Cat # 12604)
6. T75, T182 tissue culture treated flasks
7. Graduated pipets (2, 5, 10, 25, 50 ml)
8. DMSO (Fisher; Cat # BP-231-100)
9. Cryovials (Sarstedt; Cat # 72-694-006)
10. TC20 cell counter (Bio-Rad)
11. Counting Slides (Bio-Rad; Cat # 145-0011)
12. 0.40% Trypan Blue Dye (CORNING; Cat # 145-0013)
13. *all –trans–* Retinoic acid (Sigma, Cat # R2625)
14. Microscope

Note: Dissolve the *all –trans–* Retinoic acid in 100% of DMSO to make a 20 mM stock solution. Aliquot 100 or 150 µL per vial. Keep in dark and stored in -20 ^{0}C freezer.

Procedure

Differentiation

Note: Volume (50 ml per flask) used in this protocol is for 182 cm^2 flasks.

1) Propagate cells until density reaches about 70% confluence.
2) Remove and discard culture medium.

3) Add 50 ml of growth medium containing all 15 µL trans-retinoic acid stock solution (final concentration is 6 µM).

Note: Turn off the light in the TC hood when adding the retinoic acid.

4) Cover the flask(s) with aluminum foil. Continue to culture for 48 hours.

Harvest

1) Remove and discard culture medium.
2) Wash cells with room temperature (or warm) 1X PBS. (To remove all traces of serum that contains trypsin inhibitor.)
3) Add 10 ml (T182) of TrypLE and return to incubator for 7-15 minutes, or until cells detach.
4) 10-13 ml (T182) of complete growth medium and separate the cells by gentle pipetting.

Note: To avoid clumping DO NOT agitate the cells by hitting or shaking the flask while waiting for the cells to detach.

5) Examine viability and count cells using trypan blue staining (SOP).
6) Collect certain number of cells for your application.