

## Cell culture protocol for SH-SY5Y neuroblastoma cells (human, ATCC# CRL-2266)

Growth medium: is a 1:1 ratio of ATCC-formulated MEM and F12

- Invitrogen MEM (# 11095-080 with L-Glutamate)

Additives:-

1. 1x Non Essential amino acids [(100x stock, 1:100dilution)- Cell Gro#25-025-CI]
  2. 1mM Sodium Pyruvate [(100mM sol., 1:100 dilution)- Cell Gro#25-000-CI]
  3. 1.5g/L Sodium bicarbonate [(75g/L stock, 1:50 dilution)- Cell Gro#25-035-CI]
- Mix 1:1 with F12 media (Invitrogen# 11765-054)
  - Add 10% FBS (Gemini)
  - Add 1% Antibiotic/antimycotic

Subculturing: Even though this cell line can form floating clusters of neuroblasts, mostly these cells grow as adherent cells under the conditions described in this protocol.

- 60-80% confluent cultures are used for sub-culturing; before trypsinization, remove complete growth media and wash the cells with 1xDPBS.
- Incubate the cells with 3ml of 0.05% trypsin-EDTA (for 75cm<sup>2</sup> flask) for 5 minutes at 37°C.
- Inactivate the trypsin by adding 500ul of FBS, and triturate the cells using pipette.
- Wash the cells twice by centrifugation at 1500 rpm for 5 minutes (10ml 1xDPBS each time).
- Seed the cells (1x10<sup>6</sup> cells/mL) in fresh culture media and incubate at 37°C in 5% CO<sub>2</sub> incubator.
- Within 3-4 days, cells will become 60-80% confluent and can be used for further experiments.
- Viable cells are preserved in 10% DMSO containing growth medium in a liquid nitrogen container.