Standard Operating Procedure @ CNR- IREA

PROTOCOL	**SH-SY5Y cell culture conditions and handling**
DATE	08/06/2023
AUTHOR(S)	Mariateresa Allocca, Anna Sannino, Stefania Romeo, Maria Rosaria Scarfi, Olga Zeni
REVISED BY	Maria Rosaria Scarfi, Olga Zeni
APPROVED BY	Olga Zeni

Index

1. Purpose

This procedure describes the materials and the protocols used for maintenance and storage of SH-SY5Y cell line.

2. Background

Human neuroblastoma SH-SY5Y cell line was obtained from a metastatic bone tumor of a 4-year-old cancer patient. Frozen cryovial ($6.4x10^6$ cells) was purchased from ATCC (Cat. No. CRL2266, Manassas, VA, USA) and arrived to CNR-IREA on 31 August 2018. Upon arrival, the cells were

amplified, then some stocks were prepared according to the freezing procedure and stored in liquid nitrogen (master bank of cells at passage 3-4). A working bank of SH-SY5Y cells was established from a master bank vial in order to control the number of cell passages for NextGEM experiments.

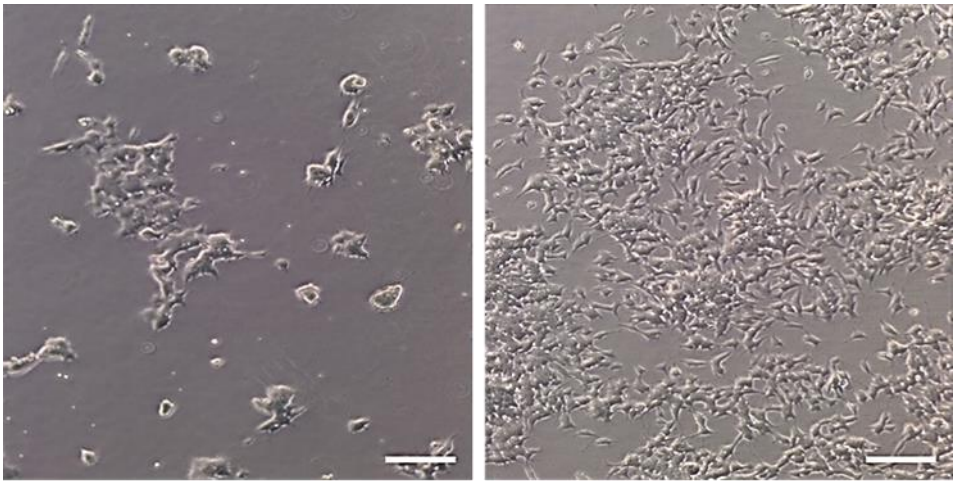

Figure 1: SH-SY5Y cell line as appeared at different growth densities at CNR-IREA lab. Inverted microscope images, scale bar: 100 µm.

The SH-SY5Y cells grow partially in suspension and mostly in adhesion (figure 1), with adherent cells weakly attached. Cultures grow as clusters (figure 2) of neuroblastic cells with multiple, short, fine processes (neurites).

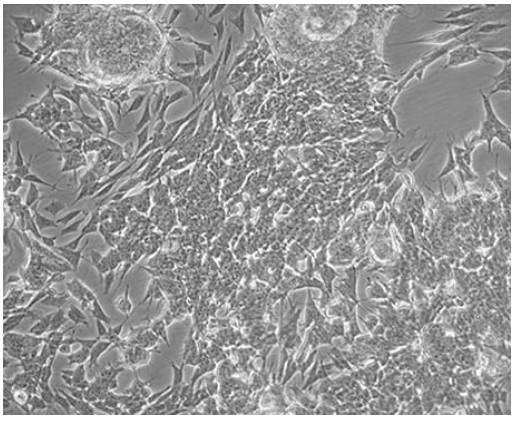

Figure 2: SH-SY5Y cell clumps.

The cell size, measured with Luna II cell counter, varies between 10-11.5 µm. The doubling time depends on the number of cells at seeding and is between 48 and 72 hours.

3. Procedure

The reagents and materials used are sterile and all the procedures are performed under a laminar flow cabinet.

3.1. Equipments

- Cell culture incubator (Thermo Scientific Forma, Model 311)
- Laminar flow cabinet (GELAIRE, BH24TG)
- Water bath (Grant Instruments, J SUB)
- Inverted microscope (Leica, DM IL)
- Refrigerated centrifuge (Thermo Electron, PK 131 R)
- Automated cell counter (Logos Biosystems, Luna II)
- Liquid nitrogen container (MVE XC 47/11-6)

3.2. Materials

- 35 mm cell culture dish (Corning, cod. 430165)
- 100 mm cell culture dish (Corning, cod. 430167)

3.3. Reagents

- DMEM (Dulbecco's Modified Eagle Medium), supplemented with 4.5 g/L glucose, sodium pyruvate and sodium bicarbonate (Microgem, cod. AL007) is stored at +4°C
- Fetal Bovine Serum (Microgem, cod. RM10432) is stored at -20°C
- 200 mM GlutaMAX™ Supplement (Gibco, cod. 35050-038) is stored at +4°C
- 100X Penicillin-Streptomycin solution (Himedia, cod. A001) is stored at -20°C
- 1X PBS (Dulbecco's Phosphate Buffered Saline) w/o Calcium w/o Magnesium (Microgem, cod. TL1006) is stored at +4°C
- Trypsin – EDTA 1X in solution w/o Calcium w/o Magnesium w/ Phenol Red (Microgem, cod. L0930) is stored at -20°C
- DMSO (Dimethylsulphoxide, LABSCAN, cod A3534) is stored at room temperature (RT)
- Trypan blue stain, 0.4% (Logos Biosystems, cod. TB0BJC2301) is stored at RT

3.3.1. Complete medium preparation

The SH-SY5Y culture medium is composed by DMEM supplemented with 10% heat inactivated FBS, 2 mM GlutaMAX™ Supplement, 1X Penicillin-Streptomycin solution.

For 100 ml complete medium: add 10 ml FBS, 1 ml GlutaMAX™ Supplement and 1 ml Penicillin-Streptomycin to 88 ml DMEM. The culture medium can be stored at 4°C for 1-2 weeks.

3.3.2. Cryoprotective medium preparation

The SH-SY5Y cells are frozen in DMEM complete medium with 5% DMSO.

For 10 ml cryoprotective medium: add 0.5 ml DMSO to 9.5 ml complete medium.

3.4. Subculturing procedure

Before splitting:

- Warm trypsin and complete medium to 37°C
- Label tubes and dishes with cell name, passage and date

Splitting:

The following volumes are referred to 100 mm cell culture dish

a. Remove the culture medium and wash the cells with 4 ml PBS

b. Add 2 ml trypsin and incubate for 5 minutes at 37°C

c. Check the detachment of cells and resuspend them in 5 ml complete medium

d. Transfer cells into centrifuge tube and spin at 300 g for 5 minutes

e. Discard the medium and resuspend the cell pellet in 4 ml fresh complete medium

f. Collect an aliquot to count the cells before dispensing the required amount into new dish containing 10 ml of fresh medium

Note:

- For maintenance: split SH-SY5Y cells once a week and seed $5x10^6$ cells in 100 mm cell culture dish
- For the experiments: use SH-SY5Y cells for a maximum of 11 passages

3.5. Freezing procedure

1. When cells are confluent, perform steps a-c described under “splitting”
2. Collect an aliquot to count the cells
3. Centrifuge the cells at 300 g for 5 minutes
4. Resuspend the cells in cryoprotective medium at a concentration of $5x10^6$/ml
5. Aliquot 1 ml of cell suspension in sterile cryovials
6. Place the cells for 1 hour at -20°C, then overnight at -80°C. Finally transfer them into liquid nitrogen for long term storage

Note: perform mycoplasma test (fluorescence DAPI test) before freezing cells

3.6. Thawing procedure

1. Take the cryovial out of liquid nitrogen and quickly thaw by hand

2. Transfer the cells into centrifuge tube containing 5 ml pre-warmed culture medium and spin at 300 g for 5 minutes
3. Discard the medium and resuspend the cell pellet in 5 ml complete medium
4. Transfer the cells to the culture dish and incubate at 37°C and 5% CO_2