DB-ALM Protocol n° 216: Cell culture protocol for the SH-SY5Y neuroblastoma cell line

Cell culture method

This protocol describes the expansion and maintenance of SH-SY5Y neuroblastoma cell line used at BioTalentum Ltd (BIOT).

Résumé

The current protocol is based on the recommended protocol of ATCC®, the supplier of the cell line, as referred in Biedler et al. 1978; and Ross et al. 1983. The protocol of BioTalentum Ltd.
(BIOT) was developed in frame of the **EU-ToxRisk** project (H2020-funded project No. 681002).

Experimental Description

Experimental/Test System:

Human SH-SY5Y neuroblastoma cell line was purchased from ATCC® (Cat. No. CRL-2266™). SH-SY5Y cell line was maintained as adherent culture and the proliferating cells are used in the assay without differentiation. Undifferentiated SH-SY5Y cells continuously proliferate, express immature neuronal markers, and lack mature neuronal markers (Pahlman et al. 1984). Undifferentiated cells are considered to be most reminiscent of immature catecholaminergic neurons (Lopes et al. 2010; Xie et al. 2010).

Discussion

In this protocol human SH-SY5Y neuroblastoma cells are maintained and proliferated for cell banking or downstream applications. This protocol describes the propagation of SH-SY5Y neuroblastoma cells for performing neurotoxicity assay with viability endpoint at BioTalentum Ltd (BIOT).
The developed acute toxicity test assay using 72 hours incubation with different concentrations of test compounds are suitable to investigate developmental toxicity of test compounds. The duration of the assay is 5 days (see **DB-ALM Protocol n° 210**). This assay is available in 120 hours chronic exposure scheme, where toxic compounds are incubated for 120 hours on SHSY5Y neuroblastoma cells (see **DB-ALM Protocol n° 211**). The duration of the 120 hours exposure assay is 7 days, the treatment period starts on next day after the plating.
Operators should be trained in cell culture and good laboratory practice. Operators can get trained within 2-3 weeks. Operators should be trained in computer handling and in using statistical programs. Otherwise no special handling is required.

Status

Known Laboratory Use:

Partners within EUToxRisk project are using this protocol (Morrison et al. 2015).

Participation in Evaluation Study:

Used in the EU-ToxRisk project (H2020-funded project No.681002).

Proprietary Issues

The distribution of the protocol or any protocol components is not limited.

Health and Safety Issues

General Precautions

Biosafety level: 1. Human cells should be cultured under aseptic conditions under BSL2 laminar flow.

MSDS Information

The Trypan Blue may cause cancer.
https://www.thermofisher.com/Trypan_blue
The MSDS of SH-SY5Y cells (ATCC® CRL-2266™) provided by ATCC® is available under MSDS_SH-SY5Y.

Abbreviations and Definitions

BSL2:	Biosafety Level 2
°C:	Celsius
DMSO:	Dimethyl Sulfoxide
DPBS:	Dulbecco's Phosphate-Buffered Saline
EDTA:	Ethylenediaminetetraacetic Acid
EMEM:	Minimum Essential Medium Eagle
FBS:	Fetal Bovine Serum
HAM's F12:	Nutrient Mixture F-12 Ham
LN2:	Liquid Nitrogen
NB:	Neuroblastoma Cell
NEAA:	Non-Essential Amino Acids
RA:	Retinoic Acid
RT:	Room Temperature
SH-SY5Y:	SH-SY5Y Human Derived Neuroblastoma Cell Line
SOP:	Standard Operating Procedure
T-25:	25 cm^2 cell culture flask
T-75:	75 cm^2 cell culture flask
V:	Volume

Last update: 27 November 2019 (Version 1.3)

PROCEDURE DETAILS, Latest Version: 27 November 2019 (Version 1.3)

Cell culture protocol for the SH-SY5Y neuroblastoma cell line
DB-ALM Protocol n° 216

Contact Details

Dr. Žofia Janštová
BioTalentum Ltd.
Aulich L. street 26., H-2100 Gödöllő
Hungary
email: zofia.janstova@biotalentum.hu

Prof. Dr. András Dinnyés
BioTalentum Ltd.
Aulich L. street 26., H-2100 Gödöllő
Hungary
email: andras.dinnyes@biotalentum.hu

Materials and Preparations

TEST SYSTEM

Human SH-SY5Y neuroblastoma cell line (originally derived from a metastatic bone tumor biopsy; Biedler et al. 1978), was purchased from ATCC® (Cat. No. CRL-2266™). The SH-SY5Y neuroblastoma cells are cultured and used in undifferentiated, continuously proliferating, morphologically neuroblast-like form, non-polarized cell bodies with few, truncated processes, in monolayer 2D culture, although cells can be differentiated into more mature, neuron-like cells with retinoic-acid (RA) induction. Cell cultures include both adherent and floating cells, but the adherent population is used in the assay, the floating cells are discarded during media changes.

EQUIPMENT

Fixed Equipment

- Automatic pipettes suitable for measuring 10 µl, 200 µl, and 1000 µl, respectively
- Cell counting chamber (Burker chamber)
- Centrifuge (e.g. Eppendorf centrifuge 5804R)
- Freezer (-20°C)
- Freezer (-80°C)
- Fridge (4°C)
- Humidified Cell Culture Incubator (37°C, 5% CO2 in air)
- Inverted microscope with phase contrast (4x, 10x, 20x objectives)
- Laminar Flow Hood (BSL2)
- Liquid nitrogen freezer
- Liquid trap
- Mr. Frosty freezing container
- Multichannel pipette suitable for measuring 300 µl
- Pipette aid
- Silicone tubing
- Vacuum pump

Consumables

- 10 ml serological pipette (BD Falcon, Cat. No. 357551)
- 15 ml sterile centrifuge tube (BD Falcon, Cat. No. 352097)
- 2 ml serological pipette (BD Falcon, Cat No. 357507)
- 5 ml serological pipette (BD Falcon, Cat. No. 356543)
- 50 ml sterile centrifuge tube (BD Falcon, Cat. No. 352099)
- 500 ml Nalgene™ Rapid-Flow™ Sterile Disposable Bottle Top Filters with PES Membrane,
- 0.2 µm pore size (Thermo Fisher Sci., Cat. No. 595-4520)
- Corning T-25 cell culture flasks with filtered cap (Merck, Cat. No. CLS430639)
- Corning T-75 cell culture flasks with filtered cap (Merck, Cat. No. CLS430641U)
- Disposable Glass Pasteur Pipettes 150 mm (Volac, Cat. No. D810)
- Filter pipette tips 10 µl (Thermo Fisher Sci., Cat. No. 94056980)
- Filter pipette tips 100 µl (Thermo Fisher Sci., Cat. No. 94056520)
- Filter pipette tips 1000 µl (Thermo Fisher Sci., Cat. No. 94056710)
- Nalgene™ Rapid-Flow™ Sterile Disposable Filter (Thermo Fisher Sci., Cat. No. 5660020)
- Nunc™ Biobanking and Cell Culture Cryogenic Tubes (Thermo Fisher Sci., Cat. No. 368632)

MEDIA, REAGENTS, SERA, OTHERS

Basic medium:

- L-glutamin (200 mM) (Thermo Fisher Sci., Cat. No. 25030024)
- Minimum Essential Medium Eagle (EMEM), (Merck, Cat. No. M2279-500ml)
- Nutrient Mixture F-12 Ham (HAM's F12), (Merck, Cat. No. N4888-500ml)
- Non-Essential Amino Acids (NEAA), (Merck, Cat. No. M7145)

Other:

- Dimethyl sulfoxide (DMSO), suitable for hybridoma (Merck, Cat. No. D2650-100ml) □ Dulbecco's Phosphate-Buffered Saline (DPBS), without Ca^{2+}, Mg^{2+} (Lonza, Cat. No. BE17-512F)
- Fetal Bovine Serum (FBS), heat-inactivated, EU-approved (Thermo Fisher Sci., Cat. No. 10500064)
- Trypsin-EDTA (Thermo Fisher Sci., Cat. No. 15400054)
- Trypan blue solution 0.4% (Thermo Fisher Sci., Cat. No. 15250061)

PREPARATIONS

Media and Endpoint Assay Solutions

Trypsin-EDTA:

- Add 1 ml 0.5% Trypsin-EDTA to 9 ml DPBS to a final concentration of 0.05 µg/ml.
- Trypsin-EDTA solution can be stored at 4°C for a maximum of 1 week.

Neuroblastoma cell (NB) medium preparation:

- Mix all the components listed in Table 1 and sterilize it by filtering through a 500 ml 0.2 µm pore size rapid filter.
- Store NB culture medium at 4°C for a maximum of 2 weeks from preparation date.

Table 1. Neuroblastoma cell (NB) culture medium.

Components of NB medium	Final concentration	V=100 ml
EMEM	45%	45 ml
HAM'sF12	45%	45 ml
FBS	10%	10 ml
NEAA (100x)	1% or (1x)	1 ml
L-glutamin (200 mM)	2 mM	1 ml

Neuroblastoma cell (NB) freezing medium preparation:

- Mix all the components listed in **Table 2**.
- Mix well and use the diluted solution immediately for freezing.

Table 2. Neuroblastoma cell (NB) freezing medium.

Components of NB cell freezing medium	Final concentration	V=10 ml
Culture medium (NB)	90%	9 ml
DMSO	10%	1 ml

Method

TEST SYSTEM PROCUREMENT

Human SH-SY5Y neuroblastoma cell line was purchased from ATCC® (Cat. No. CRL-2266TM). SH-SY5Y cell line was maintained as adherent culture and the proliferating neuroblastoma-like cells are used in neurotoxicity assay without differentiation.

ROUTINE PROCEDURES

Thawing SH-SY5Y neuroblastoma cell line:
Thawing of cryopreserved SH-SY5Y neuroblastoma cells.

- Take out 1 vial of SH-SY5Y cells ($2.5x10^6$ cells/vial) from the liquid LN2 tank and put on dry ice.
- Prepare 5 ml NB medium in a 15 ml centrifuge tube.
- Thaw the vial in 37°C water bath.
- Before moving the vial under a sterile hood clean the outer surface of the vial by spraying 70% alcohol on it then wait a few seconds till the alcohol evaporates.
- Transfer the cell suspension dropwise into the 5 ml NB culture medium, prepared previously in a centrifuge tube.
- Centrifuge the cells on RT at 1000 rpm for 3 minutes.
- Aspirate the medium.
- Resuspend the cell pellet in 4 ml NB culture medium.
- Plate the cells into a T-25 flask.
- Change the medium in every second day by aspirating the used medium and then add 4 ml of fresh medium.

Passaging SH-SY5Y neuroblastoma cells:

When the cell confluence reaches 85% the cells must be passaged. After thawing it becomes due in about 4 days. Then you split all the cells from the T-25 to a T-75 flask (seeding ratio is 1:1). By the further passages (from T-75 to T-75) the usually used splitting ratio is 1:10.

- Aspirate the medium.
- Wash the cells with DPBS.
- Add 3 ml 0.05% Trypsin –EDTA and incubate the cells for 3 min at 37°C.
- Add 6 ml NB medium to inactivate the Trypsin.
- Suspend and collect the cells in a15 ml falcon tube.
- Centrifuge the cells on 1000 rpm for 3 min at RT.
- Aspirate the medium.
- Resuspend the cell pellet in fresh medium. Split the cells in 1:10 ratio.
- Add 12 ml cell suspension per T-75 flask.

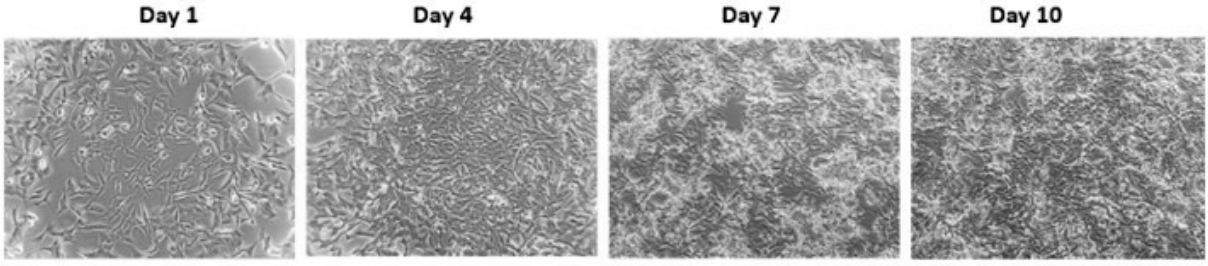

Figure 1. Morphological characteristic of SH-SY5Y neuroblastoma cells on different days after plating. Cells were plated in 150.000 cells/cm^2 density.

Freezing SH-SY5Y neuroblastoma cells:

- Aspirate the medium.
- Wash the cells with DPBS.
- Add 3 ml 0.05% Trypsin –EDTA and incubate the cells for 3 min at 37°C.
- Add 6 ml NB medium to inactivate the Trypsin.
- Suspend and collect the cells in a15 ml falcon tube.
- Centrifuge the cells on 1000 rpm for 3 min at RT.
- While the cells are in the centrifuge, count the cells using a Burker's chamber.
- Aspirate the supernatant then resuspend the cells in the right amount of freezing medium.
- Freeze $2.5x10^6$ cells/vial in 1 ml freezing medium.
- Put the cells in a freezing container and store at -80°C for one day.
- Next day transfer cells to a liquid nitrogen tank.

Cell counting (Trypan blue dye):

- Under sterile conditions take 10 µl cell suspension and add 10 µl Trypan blue dye (1:1), mix and place 10 µl in the Burker chamber.
- Cover the chamber with the appropriate coverslip.
- Count the cells under inverse microscope and calculate the cell number per ml.
- According to the calculated cell number the appropriate cell dilution can be prepared.

Bibliography

- Biedler JL, et al. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. Cancer Res. 38: 3751-3757, 1978. PubMed: 29704
- Ross RA, et al. Coordinate morphological and biochemical interconversion of human neuroblastoma cells. J. Natl. Cancer Inst. 71: 741-749, 1983. PubMed: 6137586
- Pahlman S, et al. Retinoic acid-induced differentiation of cultured human neuroblastoma cells: a comparison with phorbolester-induced differentiation. Cell Differ. 14(2):135–144.1984; PubMed: 6467378
- Lopes FM, et al. Comparison between proliferative and neuron-like SH-SY5Y cells as an in vitro model for Parkinson disease studies. Brain Res.1337:85–94. 2010. PubMed: 20380819
- Xie HR, et al. SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease. Chin Med J. 123(8):1086–1092. 2010. PubMed: 20380819