Sigma-Aldrich®

Data Sheet

SHSY-5Y Tet-ON Inducible Alpha-Synuclein Neuroblastoma Cell Line

Cancer Cell Line

Cat. # SCC291

Pack size: ≥1x10^6 viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Background

Parkinson's disease, the most common neurodegenerative movement disorder[1], is caused by the progressive degeneration of dopaminergic neurons. A signature pathology of the brains of Parkinson's patients is the buildup of misfolded aggregates of the protein α-synuclein.[2] Aggregates of α-synuclein are also implicated in Lewy body dementia and Alzheimer's disease.[3] Although study of the complete pathogenesis of neurodegenerative diseases such as Parkinson's requires animal models, cellular models that reflect distinct aspects of Parkinson's pathology are of great value for understanding the multifaceted character of neurodegenerative diseases and useful for developing targeted treatments.

The SHSY-5Y Tet-ON inducible α-synuclein neuroblastoma cell line serves as a model for both dopaminergic neuronal biology and α-synuclein pathology. SHSY-5Y cells may be induced upon treatment with retinoic acid to differentiate into neuronal-type cells, enabling studies of neuronal function and differentiation.[4] SHSY-5Y cells secrete catecholamines and thus serve as a model for dopaminergic neurons. The SHSY-5Y TetON α-synuclein inducible cell line harbors an α-synuclein construct inducible by treatment with doxycycline, thus allowing controlled overexpression of α-synuclein before or after neuronal differentiation.[5] The construct also includes a *tet* repressor, which prevents leaky expression in absence of inducer. This unique cell line is an excellent model for both the basic cellular physiology of Parkinson's disease and therapeutic development.

Source

The SHSY-5Y Tet-ON Inducible α-Synuclein cell line is stably transfected with a construct containing an alpha-synuclein cDNA cassette and CMV promoter under control of Tet-ON response element.[5] The parental SHSY-5Y cell line was derived from a metastatic neuroblastoma of a 4-year-old female.[6]

Short Tandem Repeat (STR Profile)

D3S1358:	15,16	D13S317:	11
D7S820:	7, 10	D16S539:	8, 13
vWA:	14, 18	TH01:	7, 10
FGA:	23.2, 24	TPOX:	8, 11
D8S1179:	15	CSF1PO:	11
D21S11:	31, 31.2	Amelogenin:	X
D18S51:	13, 16	Penta D:	10, 12
D5S818:	12	Penta E:	7, 11

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operates as MilliporeSigma in the US and Canada.

EMD Millipore Corporation, 28820 Single Oak Drive, Temecula, CA 92590, USA 1-800-437-7500

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Cancer cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- SHSY-5Y Tet-ON Inducible α-Synuclein Neuroblastoma cells are verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services
- Cells tested negative for infectious diseases against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

SHSY-5Y Tet-ON Inducible α-Synuclein Neuroblastoma cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

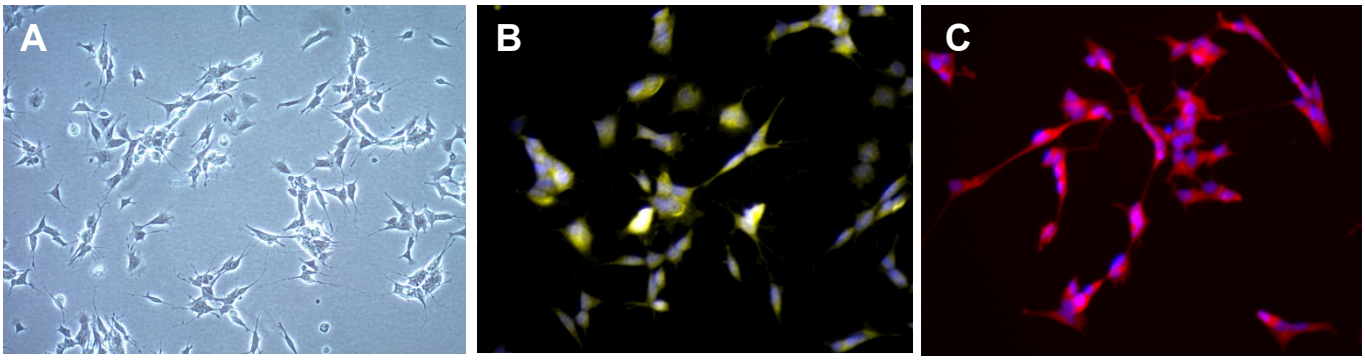

Figure 1. Bright-field image of SHSY-5Y Tet-ON Inducible α-Synuclein cells two days (A) after thaw. Cells express α-synuclein, oligomer-specific Syn 33 (B, Sigma ABN2265) and ChAT (C, Sigma AB143).

Protocols

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating.

   Cells are thawed and expanded in SHSY-5Y TetON Expansion Medium comprising DMEM/F12 medium (Sigma DF-042) and 10% FBS (e.g. Sigma ES-009-B).
2. Remove the vial of frozen SHSY-5Y Tet-ON cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.

5. Using a 10 mL pipette, slowly add dropwise 9 mL of SHSY-5Y Tet-ON Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of SHSY-5Y Tet-ON Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. Do not allow the cells to grow to confluency. SHSY-5Y Tet-ON cells should be passaged at ~70-80% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of SHSY-5Y Tet-ON cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase and incubate in a 37°C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of SHSY-5Y Tet-ON Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of SHSY-5Y Tet-ON Expansion Medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

    IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

SHSY-5Y Tet-ON Inducible α-synuclein neuroblastoma cells may be frozen in SHSY-5Y Tet-ON Expansion Medium supplemented with 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Falkenburger BH, Saridaki T, Dinter E. *J Neurochem* 2016;139 (Suppl 1):121-130.
2. Balestrino R, Schapira AHV. *Eur J Neurol* 2020; 27(1):27-42.
3. Twohig D, Nielsen HM. *Mol Neurodegener* 2019; 14(1):23.
4. Agholme L et al. *J Alzheimer's Dis* 2010; 20(4):1069-1082.
5. Vasquez V, Mitra J, Perry G, Rao KS, Hegde ML. *J Alzheimers Dis* 2018; 66(2):453-460.
6. Biedler JL, Helson L, Spengler BA. *Cancer Res* 1973; 33(11):2643-2652.
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