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Maintenance & Differentiation: SHSY-5Y Neuroblastoma Cells

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Protocol status: Working **We use this protocol and it's**

working

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Abstract

This protocol details the maintenance & differentiation of SHSY-5Y Neuroblastoma Cells.

Materials

Media:

- 1. Maintenance Media: DMEM full media containing (DMEM/10% FBS/1% Pen-Strep).
- 2. Differentiation Media: Complete Neurobasal media containing
- I. Neurobasal-A (1x) Media Gibco
- II. Pen-Strep (1% final)
- III. B27-Supple (50x) Gibco-(1.0x final)
- IV. Retinoic Acid in DMSO (Stock: 10 mM); use10 μM (Final). 1μl of stock per 1ml media.

Note

Add Ingredient IV Retinoic acid freshly each time

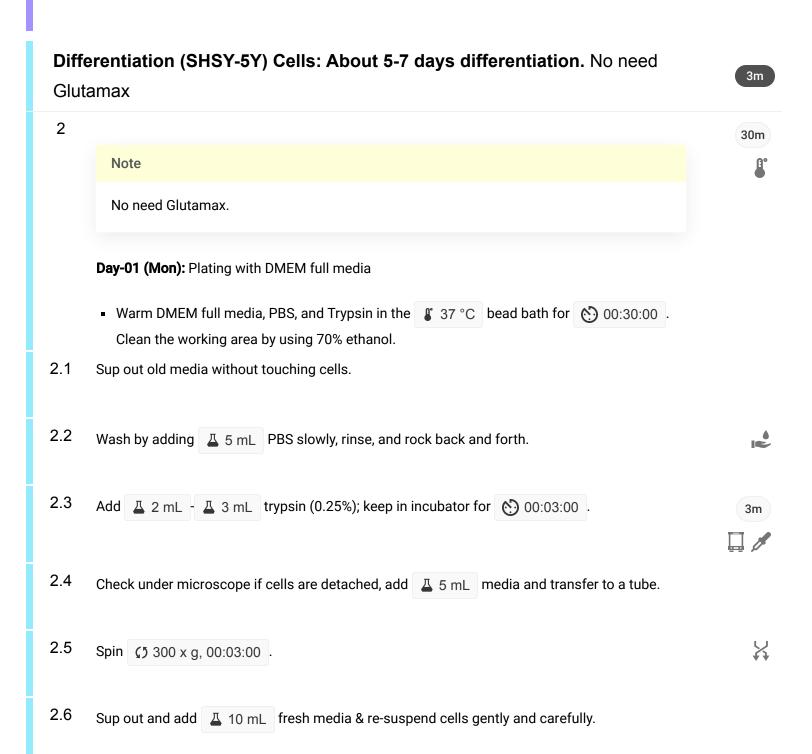
For differentiation:

- 12 Well plate (1 ml/well)
- 6 Well plate/35 mm dish (9.5 cm²-2 ml/well)
- 60 mm dish (21 cm²-3.5 ml/dish)
- 100 mm dish (56 cm²-10 ml/dish)
- 250 ml Flask for sub-culture/maintenance (10 ml) [Tissue culture flask-Greiner bio-one- Cat.No.-658 170]
- CELL CULTURE FLASK, 250 ML, 75 CM², PS, RED STANDARD SCREW CAP, CLEAR, CELLSTAR® TC, STERILE, 5 PCS. greiner bio-one Catalog #658170



Maintenance

1 For regular maintenance of SHSY-5Y cells, use DMEM full media.





- 2.7 Count cells density and split accordingly. 15,000 cells/ml for maintenance
 - Usually 1.0x10^4/ml cells for Biochem, and
 - 0.5x10⁴/ml cells for IF.

3	Dav	y-02	(Ti	ue)	١:

Replace with Complete Neurobasal Media (Without Glutamax).

Note #Add Retinoic Acid freshly

4 Day-03 (Wed):

Rest.

5 Day-04 (Thu):

Rest.

6 Day-05 (Fri):

Replace with Complete Neurobasal Media (Without Glutamax) /(Start drug treat if necessary)

Note

Add Retinoic Acid freshly

7 **Day-06 (Sat):**

Rest.

8 Day-07 (Sun):

Rest.

9 Day-08 (Mon):

Replace with Complete Neurobasal Media (No Retinoic acid)/Drug treat.



10 Day-09 (Tue):

Drug treat if necessary / Harvesting.

11 Day-10 (Wed):

Drug treat if necessary / Harvesting.

Cells harvesting:

20m 2s

12 Wash once with cold PBS.



- 13 Add cold lysis buffer.
- 14 Keep & On ice & scrap immediately in Eppendorf tube.
- 15 Sonicate (10 S on 00:00:02 off 20% Amplitude, 2 Pulses)

2s

16 Boil (100 °C , 00:10:00).



17







- 19 BCA to measure protein concentration.
- 20 Prepare with sample buffer and run WB analysis.