TransDiffer™ Human Neural Stem Cells Differentiation Kit

Cat. No. MN301

Storage: at -20°C in the dark for one year, avoid repeated freeze-thaw cycles

Description

TransDiffer™ Human Neural Stem Cells Differentiation Kit is a chemically defined and animal component-free medium for the neural induction of human pluripotent stem cells (hPSCs). The medium is optimized to an albumin-free formulation which minimizes batch-to-batch variation. *TransDiffer*™ Human Neural Stem Cells Differentiation Kit supports rapid generation of hNSCs from hPSCs in 4 days with over 90% efficiency. Human NSCs generated using this kit can be applied for basic and clinical research on human neurogenesis and neurodegenerative diseases, neurodegenerative drug screening and toxicity evaluation, and can be potentially used as effective therapies for neurodegenerative diseases.

Kit Contents

Component	MN301-01
TransDiffer™ Human Neural Stem Cells Differentiation Basal Medium	100 ml
TransDiffer™ Human Neural Stem Cells Differentiation Supplement	1 ml

Procedures

Materials required but not included

Product Name	**Catalog**
Accutase	Thermo Fisher, Cat. A1110501
Laminin	Stem Cell Technologies, Cat. 77003
L-Glutamine	TransGen, Cat. FG201-01
PBS without calcium or magnesium	TransGen, Cat. FG701-01
Poly-L-ornithine Hydrobromide (PLO)	Sigma, Cat. P4538
Y-27632	TransGen, Cat. MS101-01

1. Prepare Complete *TransDiffer*™ Human Neural Stem Cells Differentiation Medium
   Add 1 ml of thawed *TransDiffer*™Human Neural Stem Cells Differentiation Supplement and 1 ml of L-Glutamine to 98 ml of *TransDiffer*™ Human Neural Stem Cells Differentiation Basal Medium, mix thoroughly.
   Note: Store Complete *TransDiffer*™ Human Neural Stem Cells Differentiation Medium at 2-8°C in the dark for up to 1 week. Do not re-freeze.
   Aliquot *TransDiffer*™ Human Neural Stem Cells Differentiation Supplement into one-time use aliquots, store at -20°C in the dark. Do not re-freeze.
2. Coat culture vessels with PLO/Laminin
   (1) Dilute PLO with PBS (calcium and magnesium free) to a final concentration of 15 μg/ml. Dispense 250 μl of 15 μg/ml PLO solution into each well of a 24-well plate to cover the entire surface area of each well. Incubate at 37°C for 2 hours or at 2-8°C overnight. Do not allow the vessel to dry.
   (2) Aspirate PLO solution, rinse the well twice with PBS and once with DMEM/F12.
   (3) Dilute Laminin with DMEM/F12 to a final concentration of 5 μg/ml. Dispense 250 μl of 5 μg/ml Laminin solution into each well of a 24-well plate to cover the entire surface area of each well. Incubate the coated vessels at 37°C for 2 hours or at 2-8°C overnight. Do not allow the vessel to dry.
   Note: Coat culture vessels on the day of use. Aspirate the Laminin solution just before plating cells.

3. Neural induction of hPSCs

(1) Disaggregate high quality hPSCs into single cells using Accutase. Seed hPSCs in a culture vessel coated with PLO-Laminin at a density of 1×10^5 cells/ml (500 µl per well of a 24-well plate). Feed the cells with fresh hPSCs growth medium (with 10 µM Y-27632).
Note: Addition of 10 µM Y-27632 to hPSCs growth medium increases viability after single-cell passaging.

(2) When hPSC colonies cover 15%-25% of the surface area of the culture vessel, initiate neural induction.
Note: It is a critical step. Initiate differentiation at low cell confluency (less than 15%) increases cell death after neural induction.

(3) On the day 0 to day 4 of neural induction, aspirate hPSCs growth medium, add 500 µl of Complete *TransDiffer*™ Human Neural Stem Cells Differentiation Medium to a well of 24-well plates. Re-feed the culture with fresh medium every two days.
Note: Due to high cell density in the culture from day 3 onward, it is critical for cell nutrition to double the volume of Complete *TransDiffer*™ Human Neural Stem Cells Differentiation Medium or change Complete *TransDiffer*™ Human Neural Stem Cells Differentiation Medium every day.

Notes

- *TransDiffer*™ Human Neural Stem Cells Differentiation Medium enables efficient induction of hNSCs derived from hPSCs cultured in Essential 8 medium or in *TransStem*™ Chemically Defined Xeno-free Human Pluripotent Stem Cell Medium (Cat. MP101) in feeder-free conditions.
- Start neural induction with high quality hPSCs with minimal or no differentiated colonies, or the efficiency of neural induction will be reduced.
- Mycoplasma contamination of hPSCs may decrease differentiation efficiency, reduce cell growth rate and viability during neural differentiation. Use *TransDetect*® Luciferase Mycoplasma Detection Kit (Cat. FM301) or *TransDetect*® PCR Mycoplasma Detection Kit (Cat. FM311) to test cells for Mycoplasma contamination before differentiation. Use *TransSafe*™ Mycoplasma Elimination Reagent (TransMyco-1+2) (Cat. FM401) or *TransSafe*™ Mycoplasma Elimination Reagent (TransMyco-3) (Cat. FM411) to eradicate Mycoplasma from contaminated cell cultures before differentiation.
- Insufficient coating may induce cell detachment. Cell detachment from growth surface decreases the efficiency of neural induction and inhibits cell proliferation.
- Thaw *TransDiffer*™ Human Neural Stem Cells Differentiation Supplement at 2-8°C, spin down Supplement at maximum speed for a few seconds. Aliquot Supplement into single-use aliquots, store at -20°C in the dark. Do not re-freeze.
- Add culture medium against the wall of culture vessel to avoid hNSCs detachment from the growth surface.
- *TransStem*™ Human Neural Stem Cells Expansion Medium (Cat. MN101) enables superior expansion of hNSCs derived from hPSCs using *TransDiffer*™ Human Neural Stem Cells Differentiation Kit.

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