Human iPSC Derived Neural Progenitor Kit

Product Manual for the following Catalog No.

SCR131
SCC035

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

EMD Millipore's Human iPSC Derived Neural Progenitor Kit contains human neural progenitor cells (NPCs) along with optimized medium for their expansion. Human neural progenitor cells (NPCs) are derived from induced pluripotent stem (iPS) cells generated using STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) lentivirus. The viral transgenes have been removed using cell permeable TAT-Cre recombinase (Cat. No. SCR508) with excision confirmed by quantitative PCR of genomic DNA extracted from human iPSC cells. A clone from the resulting transgene free pluripotent hiPSC was preferentially differentiated to expandable neural progenitor cells using the Human ES/iPS Neurogenesis Kit (Catalog No. SCR603). The resulting hiPSC-derived NPCs proliferate as an adherent cell monolayer and greater than 80% express the appropriate neural stem cell markers, including Nestin and Sox-2. Cells are provided at passage 3 and can be expanded for a further three to five passages with ENStem-A Neural Expansion Medium (Cat. No. SCM004). Human NPCs can be further differentiated to terminal neurons by the use of the Human ES/iPS Neuronal Differentiation Medium (Cat. No. SCM111) or ENStem-A Neuronal Differentiation Medium (Cat. No. SCM017). Differentiation leads to greater than 80-90% of the cells expressing mostly neuronal markers, βIII-tubulin and MAP2. Human iPSC-derived NPCs can be used for a variety of research applications including studies of neurotoxicity, co-culture applications and screening for molecules that induce or inhibit preferential differentiation to mature neurons.

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Materials Provided

Human iPSC-Derived Neural Progenitors (Cat. No. SCC035) Store in Liquid Nitrogen		
Component	**Item No.**	**Volume**
Human iPSC Derived Neural Progenitors	SCC035	1 x 10^6 cells
ENStem-A™ Neural Expansion Medium (Cat. No. SCM004) Store at -20°C		
Components	**Item No.**	**Volume**
ENStem-A Neural Expansion Medium	SCM004a	500 mL
FGF-2, lyophilized	GF003	50 μg

Materials Required But Not Supplied

1. Tissue culture-wares and supplies.
2. Basement membrane protein extracts such as Matrigel (BD Cat. No. 356234) or ECM gel (Sigma-Aldrich Cat. No. E1270).
3. DMEM medium (Cat. No. SLM-021-B)
4. Human ES/iPS Neuronal Differentiation Medium Kit (Cat. No. SCM111)
5. ENStem-A Neuronal Differentiation Medium (Cat. No. SCM017)
6. Human Neural Stem Cell Characterization Kit (Cat. No. SCR060)
7. Phosphate-Buffered Saline (1X PBS) (Cat. No. BSS-1005-B)
8. ENStem-A™ Neural Freezing Medium (1X) (Cat. No. SCM011)
9. Accutase (Cat. No. SCR005)
10. EmbryoMax L-Glutamine Solution (100X), 200 mM (Cat. No. TMS-002-C)
11. EmbryoMax ES Cell-Qualified Ultra Pure Water, sterile H_20, 500 mL (Cat. No. TMS-006-B)
12. EmbyroMax ES Cell-Qualified Penicillin-Streptomycin Solution, 100X (Cat. No. TMS-AB2-C) (Optional).
13. EmbyroMax Dulbecco's Phosphate-Buffered Saline w/o Ca^{++} or Mg^{++}, 500 mL (Cat. No. BSS-1006-B).
14. Scepter Handheld Automated Cell Counter (Cat. No. PHCC00000) or Hemacytometer
15. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)

16. Millicell EZ SLIDE 8-well glass, sterile (Cat. No. PEZGS0896)
17. Blocking Solution (5% normal donkey serum, 5% BSA ±0.1% Triton X-100 in 1X PBS)
18. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3-conjugated (Cat. No. AP192C) and donkey anti-rabbit IgG, Cy3-conjugated (Cat. No. AP182C)
19. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution

Storage and Stability

- ENStem-A™ Neural Expansion Medium (Cat. No. SCM004) should be stored at -20°C upon receiving.
- Human iPSC-Derived Neural Progenitors Cells (Cat. No. SCC035) should be stored in liquid nitrogen immediately after receiving. Temperature fluctuations will impair the recovery of healthy cells. Do not thaw the human cells until you are ready to do the assay of choice.
- Cells have a finite life-span. Cells are provided at passage 3. Cells should be thawed into a matrigel coated T25 flask. Upon thawing, cells are at passage 4. Cells may be expanded for further 3-5 passages. For subsequent passages, it takes approximately 5-6 days for cells plated at 2 x 10^6 cells per T75 flask to be confluent.

ENStem-A Neural Expansion Medium (Cat. No. SCM004)

Description	ENStem-A Neural Expansion Medium (Cat. No. SCM004)
Kit Components	1. ENStem-A Neural Expansion Medium, 500 mL (Part No. SCM004a) 2. FGF-2, 50 µg, lyophilized (Part No. GF003)
Storage & Stability	• ENStem-A Neural Expansion Medium should be stored at -20°C until ready to use. **Upon thawing, fresh L-Glutamine (not provided) should be added for a final concentration of 2 mM to the expansion medium.** Thawed medium should be stored at 2-8°C and given a 1-month expiration dating. Dispense into aliquots to avoid repeated heating prior to each use. • FGF-2 (50 µg, lyophilized) should be reconstituted with 5 mM Tris-HCL, pH 7.6 for a final stock concentration of 100 µg/mL. Dispense into aliquots to avoid repeated freeze-thaw. Store at -20°C
Applications	Serum free, chemically defined medium to expand and maintain neural stem cell culture.

Quality Control

Each lot is tested for the following parameters:

Sterility testing: Negative for bacterial and fungal growth

pH: 7.2 ± 0.2

Media Preparation

All media components should be stored at -20°C until ready to be used. Thaw media components at 4°C overnight or at room temperature for 4 hours before assembling the complete media.

1. FGF-2 (50 µg, lyophilized) should be reconstituted with neutral buffer such as PBS or Tris-HCl at pH 7.6 for a stock concentration of 100 µg/mL. Dispense into aliquots to avoid repeated freeze-thaw. Store at -20°C for up to 6 months.

2. Add fresh L-Glutamine (not provided) to the ENStem-A Neural Expansion Medium:

   ENStem-A Neural Expansion Medium with L-Glutamine

   99 mL ENStem-A Neural Expansion Medium (Part No. SCM004a)
   1.0 mL 200 mM Glutamine (100X; Cat. No. TMS-002-C)

   ~ 100 mL Total Volume

   Mix thoroughly. Dispense into aliquots to avoid repeated heating prior to each use. Thawed medium may be stored at 2-8 for up to 1 month. FGF-2 should be added fresh to final concentration of 40 ng/mL during media exchanges every other day.

Optional: While not necessary, antibiotics may be added to the medium. If desired, add 1 mL 100X Penicillin-Streptomycin Solution (Cat. No. TMS-AB2-C, not included in the kit). While not required, the complete medium may be sterile filtered using a 0.22 µm filter (Cat. No. SCGP00525, not included in the kit).

Characterization of Cells

Each lot of Human iPSC-Derived Neural Progenitor Cells has been validated to express >80% Nestin and Sox-2-positive cells and tested negative for mycoplasma. Upon differentiation, greater than 80% of the cells express terminally differentiated neuronal markers, βIII-tubulin and MAP2. Cells are provided at passage 3 and can be expanded for further 3-5 passages. Cells may grow slowly. From time of thaw to time of passage is approximately 6-7 days.

Step 1: Preparation of Coated Plates

Table 1: Volumes recommended for coating cultureware:

CultureWare	Coating Volume (mL)	Cell Culture Volume (mL)	Surface Area (cm^2)
24 well plate	0.5 mL/well	1 mL/well	2.0
6 well plate	2 mL/well	3 mL/well	9.6
T25 flask	3 mL	5 mL	25
T75 flask	8 mL	20 mL	75

MATRIGEL COATING:

Expansion & differentiation of Human iPSC derived neural progenitor cells require culture wares that are coated with Matrigel. Below are general guidelines for the coating of 6- well plates and culture flasks with Matrigel.

1. Thaw Matrigel on ice or at 2 – 8ºC overnight. Keep on ice and use pre-cooled medium and pipettes to avoid gelling of the ECM gel. **IMPORTANT: Do not thaw Matrigel at temperatures higher than 15ºC to avoid gelling.**

2. Dilute the Matrigel 1:50 with cold DMEM medium. For example, to every 1 mL Matrigel, add 49 mL cold DMEM medium for a total volume of 50 mL. Scale accordingly to the volumes required.

3. Cover the cultureware with the recommended volumes (see Table 1). Incubate at room temperature for 1 hour or 2 – 8ºC overnight. Do not let the coated cultureware sit at room temperature for longer than 1 hour as the matrigel will start to gel. If not used immediately, store coated culture ware at 2-8ºC until ready to use.

   ***Note**: If not used immediately, Matrigel coated culturewares should be sealed with parafilm to prevent evaporation and can be stored at 2 – 8ºC for up to one week or stored frozen at -20ºC for up to 3 months.*

4. Prior to seeding the cells, bring the plate back to room temperature, remove the coating solution and add an appropriate volume of the ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2. . **IMPORTANT: Do not allow the flask to dry out.**

Step 2: Thawing Cells onto a T25-Coated Flask

Do not thaw the cells until the recommended medium and appropriately coated T25 flasks are on hand. Cells should be thawed onto a T25 flask coated with Matrigel. **It is <u>not</u> recommended to thaw to a larger sized flask.**

1. Prepare the ENStem-A Neural Expansion Medium containing L-Glutamine and 40 ng/mL FGF-2 (refer to pgs. 3 – 4 for media preparation).

2. Remove the vial of cryopreserved cells from liquid nitrogen storage and quickly thaw the cells in a 37°C water bath. Closely monitor until the cells are completely thawed. Remove the vial from the water bath as soon as cells have thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells or leave them in the water bath for too long.**

3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol or isopropanol. 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 to not introduce any bubbles during the transfer process.

5. Using a 10 mL pipette, slowly add dropwise 9 mL of ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2 (pre-warmed to 37ºC) to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of media at once to the cells. This may result in decreased cell viability due to osmotic shock.**

6. Gently mix the cell suspension by slow pipeting up and down twice. Be careful to not introduce any bubbles. **IMPORTANT: Do not vortex the cells.**

7. Centrifuge the tube at 1000 rpm for 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 a total volume of 5 mL of ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2 (pre-warmed to 37ºC). Perform trypan-blue exclusion to examine the cell number and the viability.

10. Plate the cell mixture onto a Matrigel-coated T25 flask.

11. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

12. The next day, exchange the medium with fresh ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2 (pre-warmed to 37ºC). Cells should be approximately 40-50% confluent. Exchange with fresh medium every 2 to 3 days thereafter.

13. When the cells are confluent, they can be dissociated with Accutase™ and passaged to a new flask coated with 1:50 dilution of Matrigel. For expansion, proceed immediately to Step 3 (see below). Alternatively, the cell pellet may be cryopreserved for later experiments. In the event of cryopreservation, use ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2 and 10% DMSO. It is recommended to bank the cells at 3 – 5 million cells per vial.

Step 3: Neural Progenitor Expansion with ENStem-A Medium (SCM004)

The protocol is based on the expansion of neural progenitors starting from T25 flasks. The volume should be adjusted according to the tissue culture vessel used.

1. Prepare tissue cultureware according to the section on "**Preparation of Coated Plates**". We recommend starting with T75 flasks coated with a 1:50 diluted Matrigel Solution.

2. Prepare ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2 according to the section on "**Media Preparation**" on pgs. 3 – 4.

3. When cells are approximately 95-100% confluent, they can be dissociated with Accutase™ and passaged. To passage, rinse the plate or flask once with 1X PBS and aspirate. Add sufficient amounts of Accutase to cover the surface of the flask (use 3-5 mL Accutase for T25 flasks). Incubate at 37ºC for 3 to 5 minutes.

4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.

5. Transfer the dissociated cells to a 15 mL conical tube. Add an equal amount (3 – 5 mL) of ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2 to collect any residual cells.

6. Centrifuge at 800 – 1000 rpm for 5 minutes. Discard the supernatant and resuspend the cell pellet in 2 mL ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2. Count the number of cells using a Scepter or hemacytometer. Determine cell viability using Tryphan blue exclusion.

7. Plate cells at ~3 x 10^4 cells/cm^2. This corresponds to 2 – 2.5 x 10^6 cells per T75 flask in ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2.

Step 4: Neuronal differentiation with Human ES/iPS Neuronal Differentiation Medium (Cat. No. SCM111, not provided, available separately)

The protocol is based on the terminal differentiation of Human ES/iPS derived NPCs in 24 well plates.

1. Coat 24 well plates with 1:50 diluted Matrigel Solution. Please refer to the section "**Preparation of Coated Plates**".

2. Prepare 50 mL of the Human ES/iPS Neuronal Differentiation Medium (Cat. No. SCM111, not provided, available separately), without the dibutyryl-cyclic-AMP solution.

   Human ES/iPS Neuronal Differentiation Medium (without dibutyryl cyclic –AMP)

   49.0 mL Neuronal Differentiation Basal Medium (Part No. CS211007)
   1.0 mL Neural Supplement 1 (50X) (Part No. CS210992)
   100 µL 100 mM Ascorbic Acid-2-Phosphate Solution (Part No. 2004011)
   ~ 50 mL Total Volume

   Alternatively, the ENStem-A Neuronal Differentiation Medium (Cat. No. SCM017, not provided, available separately) may also be used as the differentiation medium.

3. Before using, remove the matrigel coating solution and add 0.5 mL ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2 to each well.

4. Plate human ES/iPS derived neural progenitor cells at 150,000 cells per well of a 24 well plate in ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2. Total volume per well = 1.5 mL. At this plating density, cells should be > 70% confluent by the next day. Users may need to optimize the initial plating density if alternative multiwell plates other than 24-well plates are used. Cell attachment may vary depending upon the surface characteristics of the vessels used (i.e. glass coverslips versus tissue culture plastic). Incubate overnight at 37ºC.

   Note: Do not start the differentiation until the cells are >70% confluent. If cells are too sparse, they may not be able to survive the length of the differentiation process.

5. Evaporation may occur due to the small sample volume and the long duration of differentiation. It is thus critical to maintain high humidity during cell differentiation to avoid evaporation. We recommend filling the outer wells of the 24-well plates with sterile distilled water to help maintain the humidity within the plate. For long differentiation experiments, (>9 days), cell death may be observed if high humidity is not maintained. Minimize opening and closing of the CO_2 incubator.

6. The next day, carefully remove 50% of the medium (i.e. this corresponds to 0.75 mL volume) and replace with 0.75 mL per well of fresh Human ES/iPS Neuronal Differentiation Medium without the dibutyryl cyclic-AMP (see page 7). Total volume per well = 1.5 mL.

   Note: *Subsequent media changes should always be done at 50% volume (i.e. 0.75 mL) to avoid perturbation to cell attachment during media changes. For longer differentiation time courses that are greater than 9 days, it is critical to maintain high humidity. Some cell death may*

occur. Users are recommended to closely monitor the health of the culture to determine the optimal end point of differentiation. Cells are highly sensitive to changes in humidity.

7. Change with fresh Human ES/iPS Neuronal Differentiation Medium without the dibutyryl cyclic-AMP every other day for up to 6 – 9 days. βIII-tubulin expression can be observed as early as day 4 of differentiation with very little GFAP detected in the culture. Beyond 9 days, cells are very sensitive to changes in humidity and it is recommended to be very careful during media exchanges and to ensure a high volume of media are in the wells (~1.5 mL/well).

8. Cells can be fixed and stored at 2 to 8ºC in 1X PBS with 0.1% sodium azide for up to 3 months.

Step 5: Immunostaining Protocol for 24 well plate (Optional)

Human NPCs and terminally differentiated neurons can be characterized using the Human Neural Stem Cell Characterization Kit (Cat. No. SCR060, not provided, available separately).

1. After 6 – 9 days of differentiation, fix the cells by incubation in freshly prepared 4% paraformaldehyde in 1X PBS for 10 minutes at room temperature.
2. Carefully aspirate the fixative and rinse four times (5 – 10 minutes each) with 1X PBS.
3. Prepare the blocking solution (e.g. 5% BSA, 5% normal donkey serum in 1X PBS). For intracellular staining, add 0.1% TX-100 to the blocking solution to permeate the cells.
4. Carefully aspirate the 1X PBS wash and add the blocking solution. Incubate at room temperature for 2 hours or overnight at 4ºC. **IMPORTANT: Do not shake the cells.**
5. Dilute the primary antibodies to working concentrations in the appropriate blocking solutions.
6. In a separate control well, depending upon the specific antibody used, add equivalent concentrations of mouse IgG (1 mg/mL) or rabbit IgG (1 mg/mL) to 0.5 mL of the appropriate blocking solution.
7. Carefully remove the blocking solution from each well and add the appropriate diluted primary antibodies to each well. Incubate at room temperature for 4 hours, or 2 to 8°C overnight. **IMPORTANT: Do not shake.**
8. Remove the primary antibody solution. Wash the cells four times with blocking solution (5 minutes each wash).
9. Dilute secondary antibodies to 1 to 5 μg/mL concentration (1:200 to 1:1000 dilutions) with the blocking solution.
10. Remove the last wash and add the appropriate diluted secondary antibody to each well. Incubate at room temperature for 1 hour. Cover the plate with tinfoil to protect from the light.
11. Remove the secondary antibody solution. Wash 4 times (5 minutes each) with 1X PBS.
12. Prepare DAPI dye: dilute the DAPI with 1X PBS to 1 μg/mL (1:10,000 dilution).
13. Remove the last wash; add DAPI staining solution and incubate at room temperature for 15 minutes.
14. Remove the DAPI solution; wash twice with 1X PBS.

15. Visualize the cell staining with a fluorescent microscope. ***Note:*** *Be sure to use the correct filter to visualize fluorescent-labeled cells.*

Data Analysis

The following are representative results obtained using the Human iPSC-Derived Neural Progenitor Cells (Cat. No. SCC035).

	CT	CT	Delta CT
	hGAPDH-pro	WPRE	WPRE-hGAPDH-pro
hiPSC-LoxP Clone #3	24.51	34.38	9.87
hiPSC-LoxP Clone #4	25.3	25.04	-0.26
H9 cells	25.71	34.74	9.03
NTC	N/A	33.97	N/A
Pre-iPS Control	25.7	25.5	-0.21

Table 2: Excision of Viral Transgene using TAT-Cre Recombinase
Human iPS cells were generated using STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) lentivirus. Human iPS clones were isolated, expanded and the viral transgenes removed using cell permeable TAT-CRE (Cat. No SCR508). Excision was confirmed by quantitative PCR of genomic DNA extracted from the human iPS clones. Cell pellets were lysed using EpiCentre's QuickExtract™ DNA Extraction Solution (EpiCentre Cat. No. QE0905T)) and used directly for quantitative PCR analysis. Two primer sets were used; one to detect human GAPDH promoter region (hGAPDH-pro) and the other to detect the viral WPRE sequence. Human iPSC clones that passed all four of the following criteria were deemed excised and transgene free:

1) CT value of hGAPDH-pro ideally needs to be less than 25 to ensure sufficient DNA in each sample.
2) The CT value of WPRE for the non-template control (NTC) sample had to be larger than 30 (This primer set formed self-dimers).
3) CT value of WPRE had to be equal or greater than those from the NTC or negative control (i.e. H9 cells).
4) Delta CT (WPRE-hGAPDH-pro) value had to be equal or greater than 5.

Sample hiPSC-LoxP Clone #3 (highlighted in yellow) passed all four excision criteria and was further expanded and preferentially differentiated to neural progenitor cells using the Human ES/iPS Neurogenesis Kit (Cat. No. SCR603).

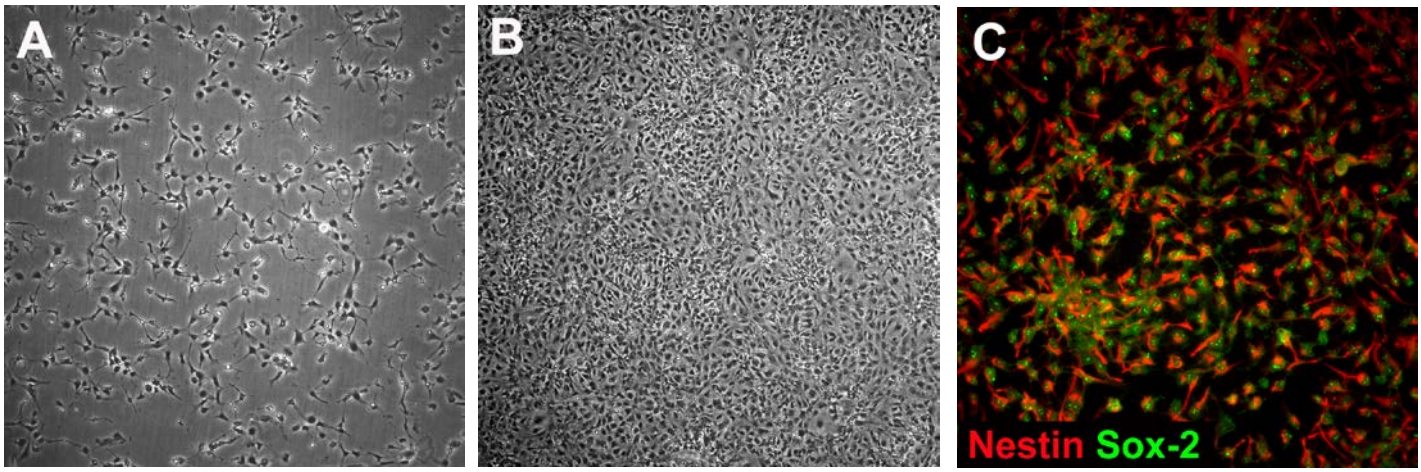

Figure 1. Human iPSC-derived Neural Progenitor Cells are provided at p3 and upon thawing, becomes p4. Cells were thawed on a Matrigel (1:50 dilution) coated T25 flask in 5 mL of ENStem-A Neural Expansion Medium containing 40 ng/mL FGF-2. (**A**). One day post-thaw. (**B**) Cells are >95% confluent at time of passage. (**C**) Majority of the neural progenitor cells express neural stem cell markers, Nestin and Sox-2.

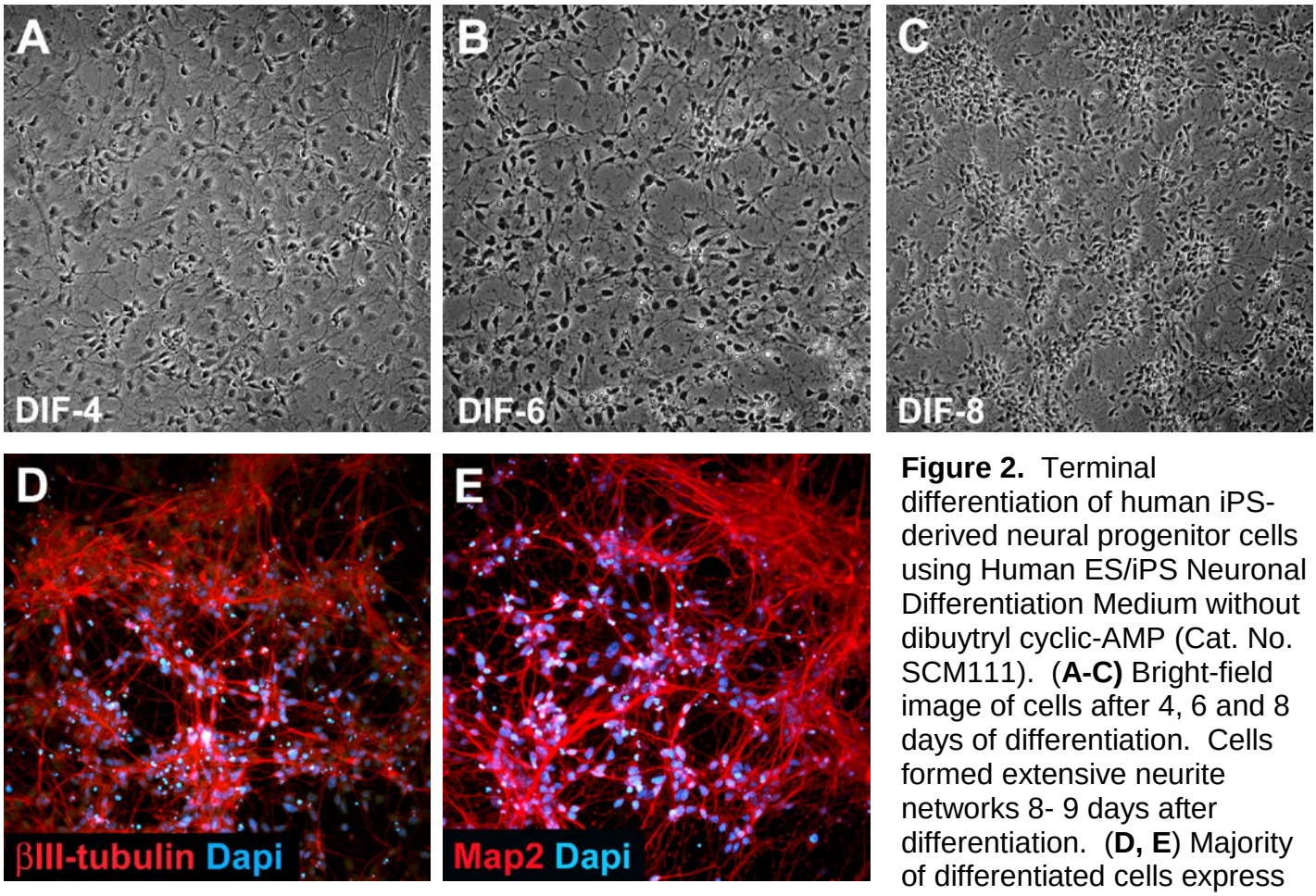

Figure 2. Terminal differentiation of human iPS-derived neural progenitor cells using Human ES/iPS Neuronal Differentiation Medium without dibuytryl cyclic-AMP (Cat. No. SCM111). (**A-C)** Bright-field image of cells after 4, 6 and 8 days of differentiation. Cells formed extensive neurite networks 8- 9 days after differentiation. (**D, E**) Majority of differentiated cells express neuronal markers, βIII-tubulin and MAP2.

Troubleshooting

Potential Problems	Possible Cause	Experimental Suggestions
Poor or no cell attachment	Expired or poor quality medium due to incorrect storage of medium (i.e. medium left at 37^{o}C or room temperature for too long).	Use medium that is within the expiration dating. Once supplemented with growth factors, aliquot into working volumes, date and keep aliquots in recommended storage conditions. Only warm up media aliquots that are necessary for the experiment. Do not warm media for more than 15-30 minutes.
	Poor ECM coating	Make sure that the coating solution completely covers the surface of the tissue cultureware. Refer to Table 1, pg 4 for recommended coating volumes. ECM coated tissue culturewares should never be allowed to dry out. After coating, use immediately or store with coating solultion at recommended temperature.
Poor cell growth	Expired or poor quality medium due to incorrect storage of medium (i.e. medium left at 37^{o}C or room temperature for too long)	Use medium that is within the expiration dating. Once supplemented with growth factors, aliquot into working volumes, date and keep aliquots in recommended storage conditions. Only warm up media aliquots that are necessary for the experiment. Do not warm media for more than 15-30 minutes.
	Contamination	Look for signs of bacteria or fungal contamination. Restart the culture with fresh cells and medium. Once the medium is supplemented with growth factors, aliquot into working volumes, date and keep aliquots in recommended storage conditions.
High cell detachment during differentiation	Media changes are too rough and disrupt cell attachment	Do not use vacuum to aspirate. During media changes, replace 50% volume. The remaining volume is necessary to buffer against cell

		perturbation during media exchanges.
High cell death during differentiation	Low humidity during differentiation	Fill the outer wells of the 24-well plates with sterile distilled water to help maintain the humidity within the plate
	Poor ECM coating	Make sure the coating solution is fresh and evenly distributed over the surface of the tissue cultureware. Refer to the table on pg. 4 for recommended coating volumes.
	ECMs other than recommended ECM is used	Matrigel has been determined to be optimal for cell attachment during long differentiation processes. Poly-L-ornithine and laminin may be used, but users may need to further optimize. Typically, the longer the differentiation, the higher the concentration of laminin may be required.
Poor antibody staining	Inadequate volume of antibody, inaccurate antibody titration or lot to lot variations in antibodies.	If species specificity is unknown, titrations may be necessary to obtain the optimal staining dilution. EMD-Millipore's Human Neural Stem Cell Characterization kit (Cat. No. SCR060) is recommended.

FAQs

1. What tissue culture flask should I thaw the cells onto?

One vial of cells should be thawed and plated onto a Matrigel coated T25 flask. It is not recommended to thaw to a larger sized flask.

2. The cells are provided at what passage?

Cells were banked at passage 3. Upon thawing, the cells are at passage 4.

3. How many times can I passage the cells?

Upon thawing, cells are at passage 4. Cells may be expanded for further 3-5 passages.

4. At what confluence should I passage the cells?

Cells should be passaged when they have reached >95% confluence.

5. What is the optimal splitting ratio?

The optimal seeding density is 3 x 10^4 / cm^2. This corresponds to ~ 750,000 cells on an appropriately coated T25 flask or 2– 2.5 x 10^6 cells on an appropriately coated T75 flask.

6. Can I freeze my cells?

Yes. We recommend using ENStem-A Neural Freezing Medium (Cat. No. SCM011)

7. Can I use my media instead?

The ENStem-A Neural Expansion Medium is recommended. Other media have not been tested. If other culture media are used, cells should be extensively characterized by user to ensure that they retain the correct phenotype and staining characteristics.

8. Can I use the ENStem-A Expansion Media or Human ES/iPS Neuronal Differentiation Media kits to expand, culture and differentiate neural progenitor cells that I have obtained from another vendor or have isolated myself?

No. These media were optimized specifically for EMD Millipore's Human ES and iPS-Derived NPCs. Other cell types have not been tested. If user is contemplating the use of other cell types, it is recommended that they use EMD Millipore's Human iPSC-Derived NPCs as a positive control to compare the effects of other cell types cultured on the media.

9. Can I use my own growth factors to differentiate?

Growth factors other than the ones provided in the kit have not been tested. If user is contemplating the use of other growth factors, it is recommended that they use the media kit provided as a positive control to compare effects of other growth factors and cytokines on human iPSC-derived NPC expansion and differentiation.

10. Can I use a different ECM to coat?

ECMs other than the ones outlined in the kit have not been tested. If the user is contemplating the use of other ECMs, it is recommended that they use the reagents and ECMs outlined in kit as a positive control to compare effects of other ECMs on human iPSC-derived NPC expansion and differentiation.

References

1. Chambers, S.M., Fasano, C.A., Papapetrou, E.P., Tomishima, M., Sadelain, M., and Studer, L. (2009). Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat. Biotechnol*. **27**: 275–280.3.

2. Li, W., Sun, W. S., Zhang, Y., Wei, W., Ambasuhan, R., Xia, P., Talantova, M., Lin, T., Kim, J., Wang, X., Kim, W. R., Lipton, S. A., Zhang, K., and Ding, S. (2011). Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors. *Proc. Natl. Acad. Sci. USA* **108**: 8299-8304.

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