Human Oligodendrocyte Differentiation Kit

Product Manual for the following Cat Nos.
SCR600
SCM106
SCM107

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

EMD Millipore's Human Oligodendrocyte Differentiation Kit contains Human Oligodendrocyte Progenitor Cells (OPCs) along with optimized media for their expansion and spontaneous differentiation into mature oligodendrocytes. Human Oligodendrocyte Progenitor Cells (OPCs) are derived from NIH approved H9 human embryonic stem cells (hESCs). These hESC-derived OPCs proliferate as an adherent cell monolayer and greater than 70% express the appropriate early-to-intermediate oligodendrocyte markers, including O4, NG2, Olig2, Sox10 and GalC. Upon growth factor withdrawal, Human OPCs spontaneously differentiate into mature oligodendrocytes expressing terminally differentiated markers including proteolipid protein (PLP), myelin basic protein (MBP), and myelin oligodendrocyte glycoprotein (MOG). Spontaneous differentiation leads to approximately 30% of the cells expressing PLP, MBP and MOG while the remaining 70% express mostly neuronal markers, MAP2 and βIII-tubulin with very little to no astrocytes (<5% GFAP-positive cells) observed. Human OPCs 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 oligodendrocytes.

Kit Components

1. Human Oligodendrocyte Progenitors (OPCs): (Cat. No. CS204496) One vial containing >5 x 10^5 viable cells, cryopreserved. Store in liquid nitrogen.

2. Human OPC Expansion Media Kit: (Cat. No. SCM107)
    - Human OPC Basal Medium (Part No. CS204464) One 100 mL bottle. Store at -20°C.
    - Neural Supplement 1 (50X) (Part No. CS210992). One 2.5 mL bottle. Store at -20°C.
    - bFGF, Recombinant Human (Part No. GF003-10UG). One 10 µg vial. Lyophilized powder. Store at -20°C.
    - OPC Expansion Supplement A (PDGF-AA), 2500X (Part No. CS204471). Lyophilized powder. Store at -20°C.
    - OPC Expansion Supplement B (NT3), 2500X (Part No. CS204470). Lyophilized powder. Store at -20°C.
    - Reconstitution Buffer 1, For Supplement A (Part No. CS204469). One 250 µL vial. Store at -20°C.
    - Reconstitution Buffer 2, For Supplement B and bFGF (Part No. CS204468). One 500 µL vial. Store at -20°C.

3. Human OPC Spontaneous Differentiation Media Kit: (Cat. No. SCM106)
    - Human OPC Basal Medium (Part No. CS204464). One 100 mL bottle. Store at -20°C.
    - Neural Supplement 1 (50X) (Part No. CS210992). One 2.5 mL bottle. Store at -20°C.

Human OPC Expansion Media Kit (Cat. No. SCM107)

Description	Human OPC Expansion Media Kit (Cat. No. SCM107).
Kit Components	Human OPC Basal Medium (Part No. CS204464). One 100mL bottle. Store at -20°C. Neural Supplement 1 (50X) (Part No. CS210992). One 2.5 mL bottle. Store at -20°C.bFGF, Recombinant Human. (Part No. GF003-10UG). One 10 μg vial. Lyophilized powder. Store at -20°C. OPC Expansion Supplement A (PDGF-AA), 2500X (Part No. CS204471). Lyophilized powder. Store at -20°C. OPC Expansion Supplement B (NT3), 2500X (Part No. CS204470) Lyophilized powder. Store at -20°C. Reconstitution Buffer 1 (Part No. CS204469) One 250 μL vial. Store at -20°C. Reconstitution Buffer 2 (Part No. CS204468) One 500 μL vial. Store at -20°C.
Storage & Stability	Store at -20°C, good for 4 months from date of receipt when reagents are handled and stored appropriately. **Please note:** The supplemented Human OPC Expansion Complete Media should be stored at 2 to 8°C for up to 4 weeks.
Applications	Human OPC Expansion Media is a defined, serum-free medium that has been optimized for the culture and expansion of human ES derived oligodendrocyte progenitor cells. Cells double every 48 hours when cultured in Human OPC Expansion Complete Media. It is not known whether the media can support the culture of primary oligodendrocyte progenitor cells isolated from brain tissues.

Quality Control

Each lot is tested for the following parameters:

Sterility testing:	Negative for bacterial and fungal growth
pH:	7.0-7.8
Osmolality:	270-310mOsm

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. Resuspend each vial of lyophilized OPC Expansion Supplements below in the following reconstitution buffers (provided). Centrifuge the liquid suspension to collect all liquid droplets. Store on ice.

   1) Recombinant Human bFGF: Resuspend with 200 μL **Reconstitution Buffer 2.**

   2) OPC Expansion Supplement A (PDGF-AA): Resuspend with 100 μL **Reconstitution Buffer 1.**

   3) OPC Expansion Supplement B (NT3): Resuspend with 80 μL **Reconstitution Buffer 2.**

2. To 100 mL of the Human OPC Basal Medium (Part No. CS204464), add 2 mL Neural Supplement 1 (50X) and 40 μL each of reconstituted Recombinant Human bFGF and OPC Expansion Supplements A and B.

Human OPC Expansion Complete Media (~100 mL):

100 mL Human OPC Basal Medium (Part No. CS204464)
2 mL Neural Supplement 1 (50X) (Part No. CS210992)
40 μL Recombinant Human bFGF (50 μg/mL) (Part No. GF003-10UG)
40 μL OPC Expansion Supplement A (PDGF-AA), 2500X (Part No. CS204471)
40 μL OPC Expansion Supplement B (NT3), 2500X (Part No. CS204470)

3. Label the front of the bottle "Human OPC Expansion Complete Media" and date. Alternatively, to reduce the risk of contamination, aliquot the media into ten 10 mL aliquots and date. The supplemented Human OPC Expansion Complete Medium can be stored at 2 to 8°C for up to 4 weeks. Discard any remaining OPC Expansion Supplements that are not used.

Human OPC Spontaneous Differentiation Media Kit (Cat. No. SCM106)

Description	Human OPC Spontaneous Differentiation Media Kit (Cat. No. SCM106)
Kit Components	Human OPC Basal Medium (Part No. CS204464). One 100 mL bottle. Store at -20°C. Neural Supplement 1 (50X) (Part No. CS210992). One 2.5 mL bottle. Store at -20°C.
Storage & Stability	Store at -20°C, good for 4 months from date of receipt when reagents are handled and stored appropriately. **Please note:** The supplemented Human OPC Spontaneous Differentiation Complete Media should be stored at 2 to 8°C for up to 4 weeks.
Applications	Human OPC Spontaneous Differentiation Media is a defined, serum-free medium that supports the differentiation of human ESC-derived OPCs to mature oligodendrocytes through growth factor withdrawal. The media does not support the differentiation of primary oligodendrocyte progenitor cells isolated from brain tissues.

Quality Control

Each lot is tested for the following parameters:

Sterility testing: Negative for bacterial and fungal growth

pH: 7.0-7.8

Osmolality: 270-310mOsm

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. To 100 mL of the Human OPC Basal Medium (Part No. CS204464), add 2 mL Neural Supplement 1 (50X).

   Human OPC Spontaneous Differentiation Complete Media (~100 mL):

   100 mL Human OPC Basal Medium (Part No. CS204464)

   2 mL Neural Supplement 1 (50X) (Part No. CS210992).

2. Label the front of the bottle “Human OPC Spontaneous Differentiation Complete Media” and date. Alternatively, to reduce the risk of contamination, aliquot into ten 10 mL aliquots and date. The supplemented Human OPC Spontaneous Differentiation Complete Medium can be stored at 2 to 8ºC for up to 4 weeks.

Materials Required but Not Supplied

1. EmbryoMax ES Cell-Qualified Penicillin-Streptomycin Solution, 100X (Cat. No. TMS-AB2-C) (Optional).
2. Tissue culture-wares and supplies.
3. Basement membrane protein extracts such as Matrigel (BD Cat. No. 356234) or ECM gel (Sigma-Aldrich Cat. No. E1270).
4. DMEM medium (Cat. No. SLM-021-B)
5. Scepter Handheld Automated Cell Counter (Cat. No. PHCC00000) or Hemacytometer
6. Phosphate-Buffered Saline (1X PBS) (Cat. No. BSS-1005-B)
7. Laminin (Cat. No. CC095)
8. Poly-L-ornithine (Sigma Cat. No. P4957)
9. EmbryoMax ES Cell-Qualified Ultra Pure Water, sterile H_20, 500 mL (Cat. No. TMS-006-B)
10. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
11. Glass coverslips, Circles No. 1.5, 0.16 to 0.19 mm thick 12 mm (Fisher Cat. No. 12-545-81)
12. Blocking Solution (5% normal donkey serum, 5% BSA $\pm$0.1% Triton X-100 in 1X PBS)
13. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3-conjugated (Cat. No. AP192C) and donkey anti-rabbit IgG, Cy3-conjugated (Cat. No. AP182C)
14. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution

Storage

- Human OPC Expansion Media Kit (Cat. No. SCM107) and Human OPC Spontaneous Differentiation Media Kit (Cat. No. SCM106) should be stored at -20°C upon receiving.
- Human Oligodendrocyte Progenitors (OPCs) (Cat. No. CS204496) should be stored in liquid nitrogen immediately after receiving. Temperature fluctuations will impair the recovery of healthy cells. Do not thaw the Human OPC cells until you are ready to do the assay of choice (i.e. differentiation, myelination, etc.).
- Cells have a finite life-span. Cells are provided at passage 3. Upon thawing, cells are at passage 4. Cells should not be expanded beyond passage 6. We recommend a seeding density of 10^4 cells/cm^2 (approximately 1:4 ratio when cells are passaged at 80% confluency in T-25 flasks). Freezing the cells for later use is not recommended and we can not guarantee the performance of cells frozen at a later passage.

Characterization of Cells

Each lot of human oligodendrocyte progenitor cells (OPCs) has been validated to express >70% Sox10- and GalC-positive cells and tested negative for mycoplasma. Upon growth factor withdrawal (i.e. spontaneous differentiation), approximately 30-50% cells express terminally differentiated oligodendrocyte markers, myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG). The remaining cells (~50-70%) express mostly neuronal markers, MAP2 and βIII-tubulin with very little to no astrocytes (<5% GFAP-positive cells) observed. Human OPCs double approximately every 48 hours and have a finite lifespan of 3-4 weeks in vitro. Cells are provided at passage 3 and should not be expanded beyond passage 6.

Step 1: Preparation of Coated Plates

For Thawing and Proliferation of Human OPCs. The provided OPCs should be thawed on Matrigel-coated plates using the following recommended procedure.

1. Thaw Matrigel on ice or at 2 to 8°C overnight. Keep on ice and use pre-cooled medium and pipettes to avoid gelling of the ECM gel.

2. Dilute 1 mL Matrigel with 49 mL pre-cooled DMEM medium in a laminar flow hood. Mix thoroughly by inverting the tube a few times.

3. Coat the desired cultureware with the diluted Matrigel. Make sure the entire surface is coated. Recommended volumes for commonly used cultureware are as follows. Please note that 4-well and 8-well chamber slides are **NOT** recommended for culturing OPCs and culturewares outside of those recommended below have not been tested.

Plate Type	Coating Volume (mL)	Culturing Volume (mL)	Surface Area (cm^2)
T75 flask	8 mL	15 mL	75
T25 flask	3 mL	5 mL	25
6 well plate	2 mL/well	3 mL	9.6
24 well plate	0.5mL/well	1 mL/well	2
96 well plate	0.1 mL/well	0.2 mL/well	0.34

4. Coat at room temperature for 1 hour or at 2 to 8°C overnight. Discard the remaining diluted Matrigel solution.

5. Coated cultureware should be used immediately after coating or frozen back at -20°C for future use (for up to one month).

6. Prior to seeding the cells, remove the coating solution and rinse with 1X PBS to remove residual ECMs. Do not allow the coating to dry out.

For terminal differentiation of Human OPCs on glass coverslips: If high magnification images are required (>63X magnification), we recommend plating cells on poly-L-ornithine and laminin-coated glass coverslips placed in 24-well plates. Glass coverslips must be acid-treated before coating. Below is a protocol for acid-treatment of glass coverslips. ***Note: We do not recommend using 4-well or 8-well chamber slides for differentiation.***

1. Place 12 mm glass coverslip circles, No. 1.5 (Fisher Cat. No. 12-545-81, not provided) in a 10 cm glass petri dish or containers that are acid resistant and autoclavable.

2. Add 10 mL 1N HCl to the glass petri dish. Incubate overnight at room temperature.

3. The next day, remove the HCl solution. Rinse the coverslips with 4 x 10 mL of distilled water. Aspirate after each rinse.

4. Sterilize the cover slips by autoclaving. Air dry in an aseptic hood.

5. Using sterile tweezers, place one glass coverslip to each well of a 24-well plate.

6. Coat the 24-well plate containing acid-treated glass coverslips as described below.

For terminal differentiation of Human OPCs on 24-well and 96-well plates: For long term differentiation assays, we recommend using poly-L-ornithine and laminin coated 24-well and 96-well plates.

1. Prepare stock solutions of poly-L-ornithine (10 mg/mL) by dissolving poly-L-ornithine in sterile water. The stock solution should be stored at -20°C or -80°C.

2. Dilute the 10 mg/mL poly-L-ornithine stock solution with water to 100 μg/mL.

3. Add enough of the poly-L-ornithine solution to cover the entire surface of the tissue cultureware. Use the table on pg. 6 to determine the appropriate coating volumes for different sized tissue culturewares. Incubate for at least one hour or overnight at room temperature.

4. Thaw the laminin on ice at 2 to 8°C or overnight. Dilute the laminin with 1X PBS to a final concentration of 10 μg/mL. Set aside.

5. Remove the poly-L-ornithine solution and rinse the tissue culturewares with 1X PBS. Aspirate after the rinse.

6. Add enough of the diluted laminin (10 μg/mL) solution to cover the entire surface of the tissue cultureware. Use the table on pg. 6 for recommended volumes for different sized tissue culturewares. Incubate for at least one hour or overnight at room temperature.

7. Coated culturewares should be used immediately after coating or may be frozen back at -20°C for future use for up to one month.

8. Prior to seeding the cells, remove the laminin solution and rinse once with 1X PBS. Do not allow the coating to dry out as it may reduce the performance of the ECM.

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 not recommended to thaw to a larger sized flask.**

1. Prepare the Human OPC Expansion Complete Media (refer to pgs. 2 – 3 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 Human OPC Expansion Complete Media (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 Human OPC Expansion Complete Media (pre-warmed to 37 ^{o}C). Perform trypan-blue exclusion to examine the cell number and the viability.

10. Plate the cell mixture onto an 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 Human OPC Expansion Complete Media (pre-warmed to 37^{o}C). Exchange with fresh medium every 2 to 3 days thereafter. A T25 flask will yield approximately 2 x 10^6 cells within one week after thawing.

13. When the cells are approximately 80% confluent, they can be dissociated with Accutase™ and passaged to a new plate. **Do not freeze cells.**

Step 3: Subculturing

1. Carefully remove the medium from the Matrigel-coated T25 flask containing the 80% confluent layer of Human OPCs.

2. Apply 3 mL of Accutase and incubate in a 37°C incubator for 3 – 5 minutes.

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

4. Apply 5 mL Human OPC Expansion Complete Medium (pre-warmed to 37^{o}C) to the flask.

5. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.

6. Centrifuge the tube at 1000 rpm for 3 minutes to pellet the cells.

7. Discard the supernatant.

8. Resuspend the cell pellet in 5 mL Human OPC Expansion Complete Medium (pre-warmed to 37^{o}C). ***Note: Do not vortex the cells.***

9. Repeat steps 6 – 7 to remove any residual Accutase.

10. Resuspend the cells in 5 mL Human OPC Expansion Complete Medium.

11. Count the number of cells using a hemacytometer or a Scepter Handheld Automated Cell Counter.

12. Plate the cells to the desired density into the appropriate coated flasks, plates or wells in Human OPC Expansion Complete Media. The recommended seeding density is 1 – 2 x 10^4 cells/cm^2. For example, we typically plate the cells at 500,000 cells on an Matrigel-coated T25 flask. ***Note: Proliferating OPCs have a finite life-span and should NOT be passaged beyond passage 6.***

Step 4: Spontaneous Differentiation of Human OPCs (24 well plate)

Note: *For qRT-PCR experiments, we recommend culturing the cells in T25 flasks instead of 24-well plates. Plating density for T25 flasks should be 2 x 10^4 cells/cm^2, which corresponds to 0.5 million cells per T25 flask.*

1. Coat 24-well plates with 100 μg/mL poly-L-ornithine and 10 μg/mL laminin. If higher magnification imaging is required, place acid treated glass cover slips (12mm circles, No. 1.5) into each well of the 24-well plate before coating (please refer to Step 1: Preparation of Coated Plates, pgs 6-7)

2. Prepare the Human OPC Spontaneous Differentiation Complete Media (please refer to pg. 4 for media preparation).

3. Plate 2.0 – 4.0 x 10^4 cells per well into an appropriately coated 24 well plate in Human OPC Expansion Complete Media. This corresponds to 1 – 2 x 10^4 cells/cm^2. Total volume per well = 1 mL. At this plating density, the cells should be 20% confluent by the next day. Users may need to optimize the initial plating density. Cell attachment may vary depending upon the surface characteristics of the vessels used (i.e. glass coverslip versus tissue culture plastic).

4. Evaporation may occur due to the small sample volume. 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 longer differentiation experiments (i.e. 14 days differentiation), cell death may be observed if high humidity is not maintained. Minimize opening and closing of the CO_2 incubator.

5. Allow the cells to attach overnight. ***Important: Do not seed the cells at a high cell density as some proliferation may occur during the first week of differentiation.***

6. The next day, carefully remove approximately 80% of the medium (i.e. this corresponds to 0.8 mL volume) and replace with 0.8 mL fresh Human OPC Spontaneous Differentiation Complete Medium (Total volume = 1 mL volume per well).

   Note: . Subsequent media changes should always be done at 80% volume (i.e. 0.8 mL) to avoid perturbation to cell attachment during media changes.

7. Change with fresh Human OPC Spontaneous Differentiation Complete Medium every 3 – 4 days for 10-14 days. For 14 day differentiation experiments, it is critical to maintain high humidity otherwise, some cell death may occur. A longer time course (3 to 4 weeks) is possible although some cell detachment may occur and users are recommended to closely monitor the health of the culture to determine the optimal end point of differentiation. The maturation of oligodendrocytes may be seen as early as two weeks based on immunostaining with a PLP-1 antibody. ***Important: Do not allow the cells to dry out during media changes.***

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

Step 5: Immunostaining Protocol (for 24 well plates)

Human OPCs and their spontaneously differentiated progenies may be immunostained using the Human Oligodendrocyte Characterization Kit (Cat. No. SCR601) (not provided, available separately).

1. After two weeks of differentiation, fix the cells by incubation in 2% 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 remove the 1X PBS wash and add the blocking solution. Incubate at room temperature for 2 hours or overnight at 4°C. **IMPORTANT: Do not shake the cells.**

5. Dilute the primary antibodies to working concentrations in the appropriate blocking solutions. For optimal results, the following antibody dilutions are recommended for immunocytochemistry.

Table 1. Recommended antibody list for characterizing Human OPCs and their differentiated progenies.

	Sox10	**GalC**	**NG2**	**MOG**	**PLP/DM20**	**MAP2**	**GFAP**
Catalog No.	AB5727	MAB342	AB5320	MAB5680	MAB388	MAB3418	MAB3402
Recommended Dilution	2.5 to 5 (μg/mL)	2.5 to 5 (μg/mL)	2.5 to 5 (μg/mL)	10 (μg/mL)	10 (μg/mL)	2.5 to 5 (μg/mL)	2.5 to 5 (μg/mL)
Subcellular localization	Nucleus cytoplasmic	Surface	Surface	Surface	Surface	Intracellular cytoskeleton	Intracellular cytoskeleton

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. For example, to obtain a 1/200 dilution of mouse anti-GalC (1 mg/mL), 2.5 μL of the antibody is added to 0.5 mL volume of the blocking solution. In an adjacent control well, add 2. 5 μL mouse IgG (1 mg/mL) control antibody to 0.5 mL of the 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 dilution) 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. If using glass coverslips, mount the coverslip onto glass slides using anti-fading mounting solution (e.g. DABCO/PVA).

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

Results:

The following are representative results obtained from the culture of Human OPCs in Human OPC Expansion Media Kit (Cat. No. SCM107) and Human OPC Spontaneous Differentiation Media Kit (Cat. No. SCM106). The cells were characterized using the Human Oligodendrocyte Characterization Kit (Cat. No. SCR601).

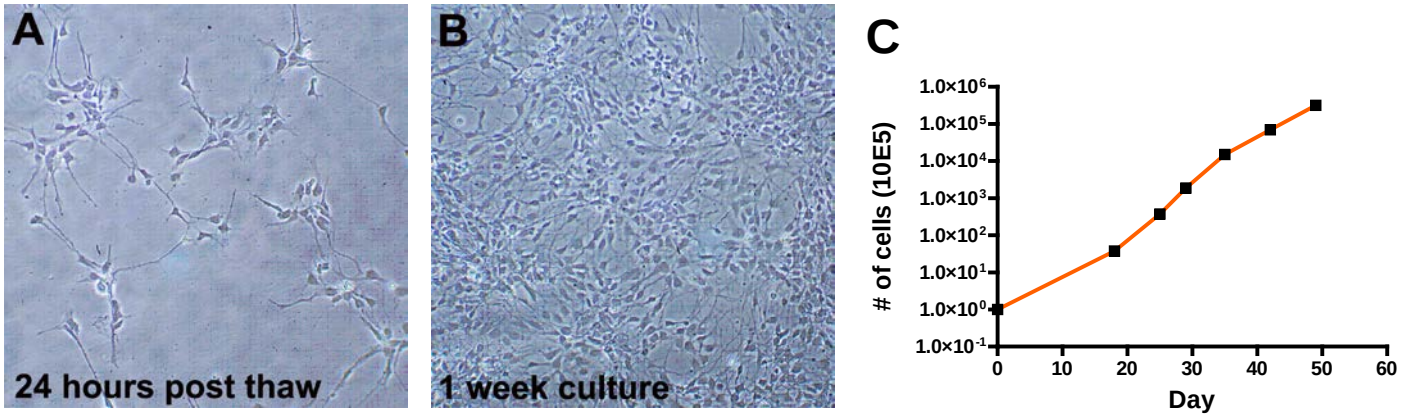

Figure 1. Bright field images of proliferating Human OPCs 24 hours post-thawing (A) and after one week in culture (B). Human OPCs double every 48 hours (C).

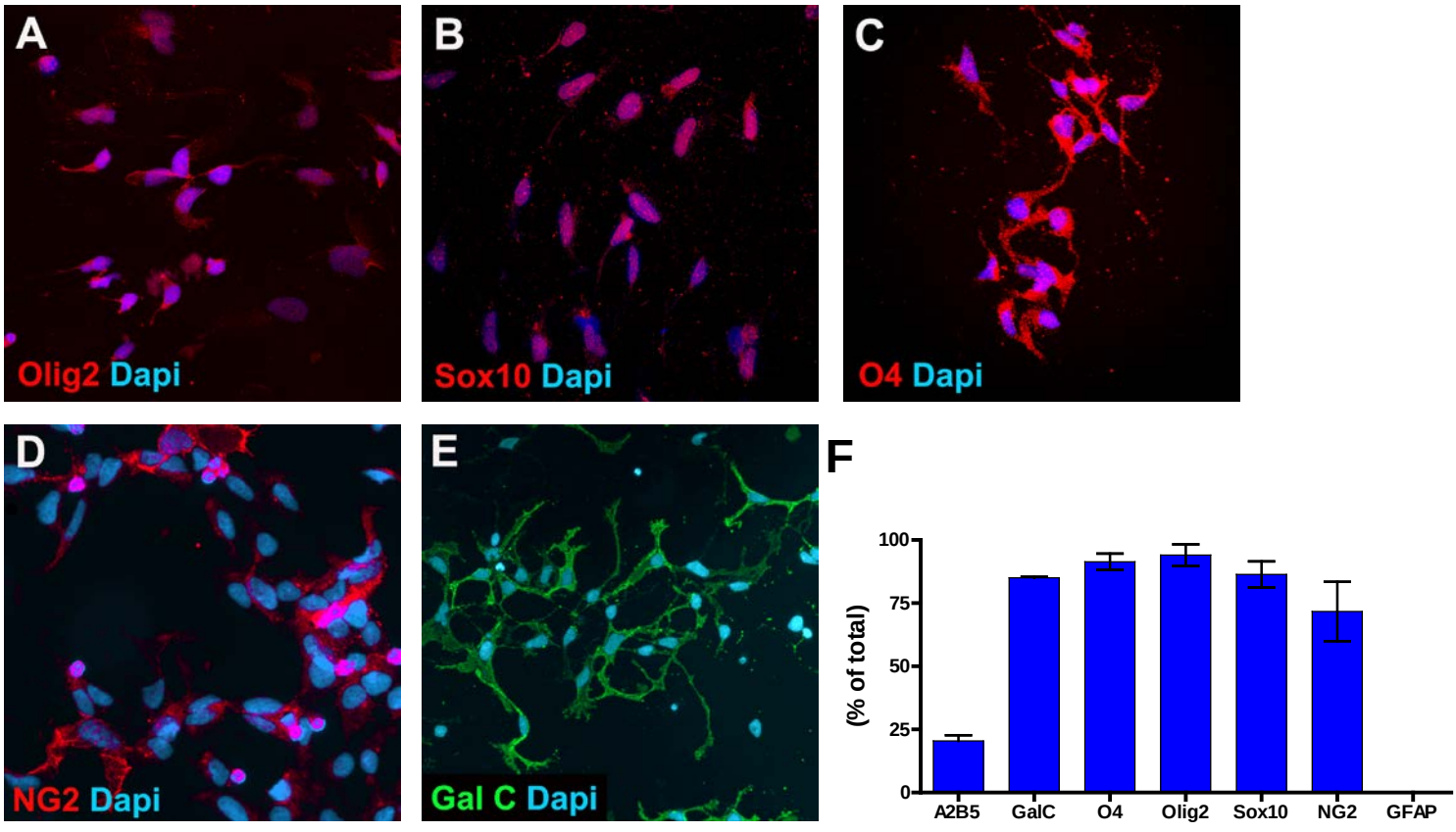

Figure 2. Human OPCs express early oligodendrocyte markers. Oligodendrocyte progenitor cells were plated at 10^4 cells/cm^2 onto poly-L-ornithine and laminin coated 24 well plates. Cells were fixed 24 to 48 hour post seeding.

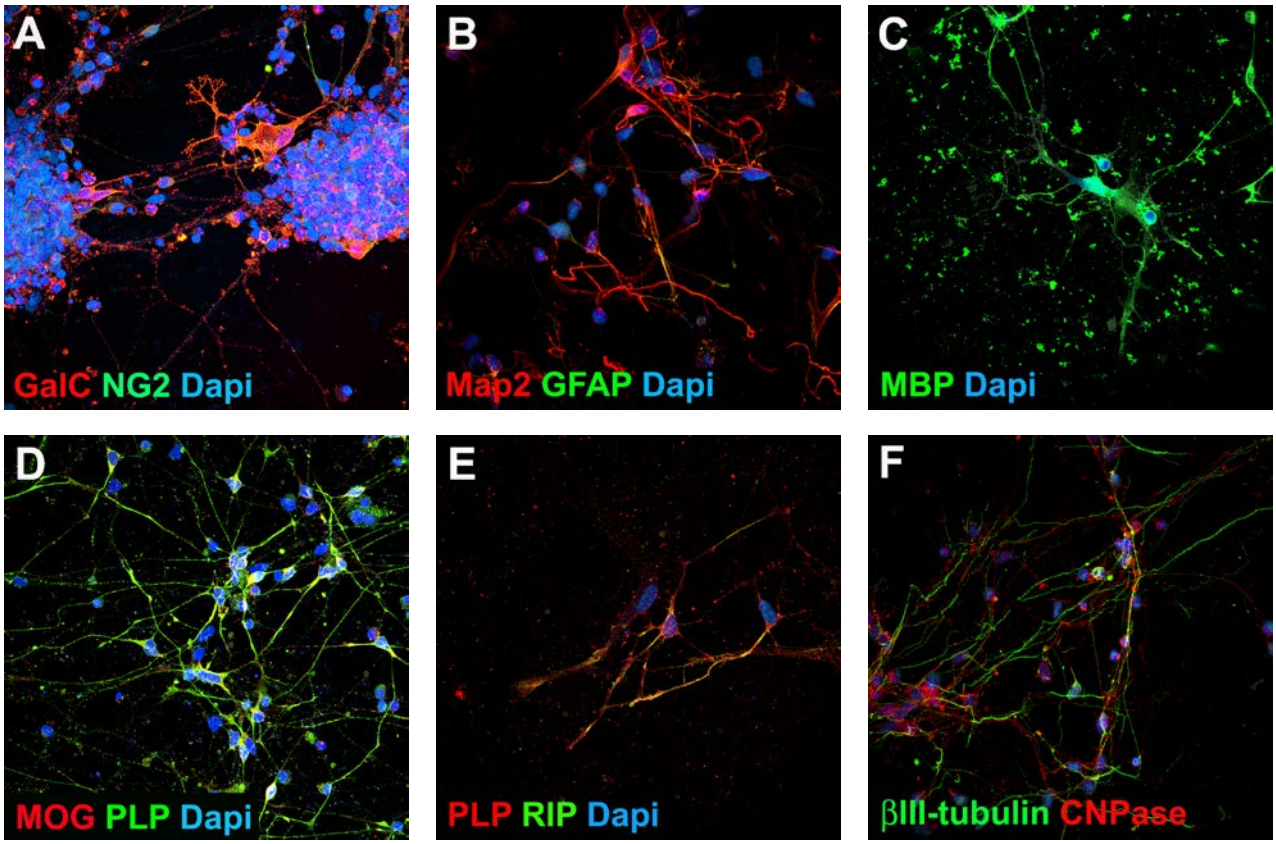

Figure 3. After 2 weeks of spontaneous differentiation, Human OPCs generate approximately 30% mature oligodendrocytes and ~50% neurons. Human OPCs were plated at 10^4 cells/cm^2 onto poly-L-ornithine and laminin coated 24 well plates in Human OPC Expansion Complete Media. Twenty-four hours post-seeding, spontaneous differentiation was initiated by media exchange with Human OPC Spontaneous Differentiation Complete Media. All images were captured using the Leica DMI-4000 confocal microscope with a 40X objective. 3D reconstruction was performed using the Leica AF software.

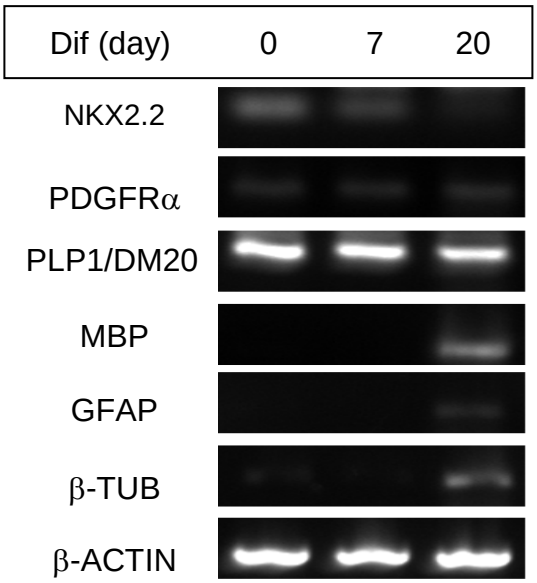

Figure 4. Gene expression analysis of Human OPC differentiation timecourse. 50 ng total cDNA was used to examine gene expressions in undifferentiated OPCs (0 day), 7 days and 20 days differentiated cells. Neural progenitor marker, NKX2.2; early oligodendrocyte progenitor markers, PDGFRα; intermediate oligodendrocyte progenitor marker, PLP-1/DM20; late oligodendrocyte progenitor marker, MBP; astrocyte marker, GFAP; neuronal marker, β-tubulin and the house-keeping gene, β-actin. Decreases in NKX2.2 expression along with a concomitant increase in MBP expression indicate that the OPCs have differentiated into mature oligodendrocytes. GFAP and β-tubulin transcripts were also weakly detected in the differentiated population.

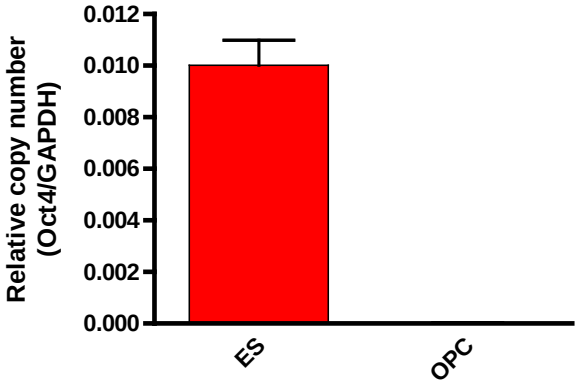

Figure 5. Two-color real-time quantitative PCR was performed to monitor the expression levels of the pluripotent gene Oct4 (SCR585) in oligodendrocyte progenitor cells. GAPDH (SCR594) was used as a house-keeping control. Oct-4 transcripts were not detected in the Human OPC cell population.

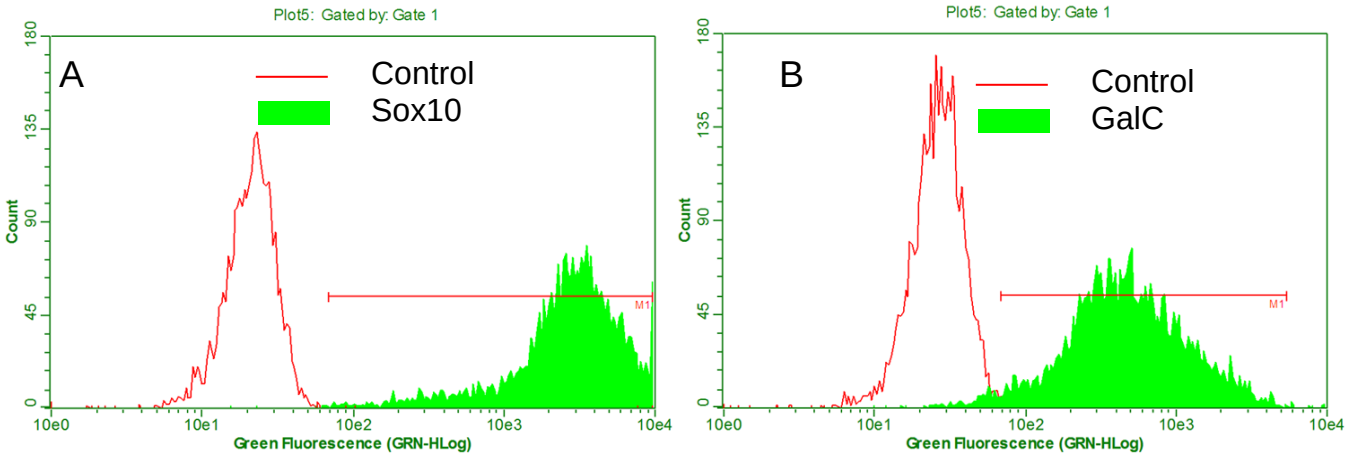

Figure 6. Flow cytometry analysis of Human OPCs. Human OPCs at passage 4 were quantitatively analyzed for Sox10 (A) and GalC (B) expressions using the Guava flow cytometer. More than 70% Human OPCs stained positive for Sox10 and GalC.

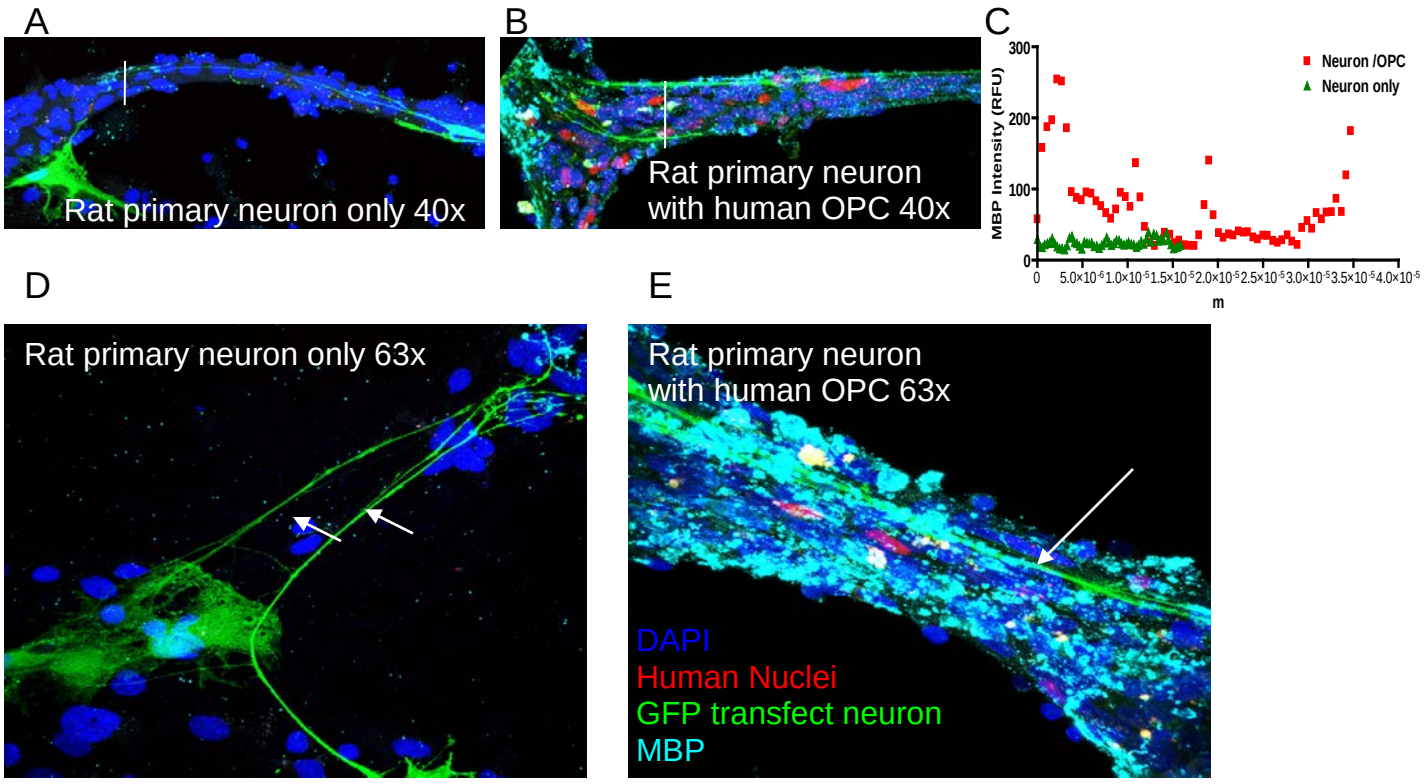

Figure 7. In vitro myelination assay. 1 x 10^5 primary E19 rat hippocampal neurons were plated onto poly-L-lysine coated cover slides in a 4 well dish with neuronal medium containing 10% heat-inactivated horse serum. Primary rat neurons were transfected with a reporter plasmid containing the constitutive chicken actin promoter driving GFP expression (pCAG-GFP). Four days after transfection, 2 x 10^4 Human OPCs (unlabeled) were co-cultured with the GFP-labeled primary neurons and the neuronal culture medium was replaced with Human OPC Spontaneous Differentiation Complete Media. The co-culture was incubated at 37°C for 21 days with media changes of the Human OPC Spontaneous Differentiation Complete Media every 2 to 3 days. Cells were fixed and stained with PE conjugated human nuclei specific antibody (Cat. No. MAB1281) and mouse anti-MBP antibody (Cat. No. 05-675, light blue). Mouse anti-MBP was detected by Cy5 conjugated goat anti-mouse secondary antibody. 3D images were acquired using confocal microscopy (Leica DMI 4000; 40x objective, 275x140μm; 63x objective, 174.6x174.6μm) and reconstructed using the Leica AF software. The line intensities of MBP (cyan) from primary neuron only (A) and primary neuron co-cultured with Human OPCs (B) were analyzed by the Leica AF software. GFP labeled axons are indicated by the white arrow. In the culture containing only rat primary neurons, there was a noticeable lack of MBP expression (D). However after 21 days of co-culture with Human OPCs, the axons of rat primary neurons appeared to be encased by MBP-positive cells (E). Analyses of the line intensities of MBP of A and B images further indicated that expression of MBP was greatly increased in the co-culture (C, red square) versus the culture containing only primary neurons (C, green triangle).

Troubleshooting

Problem	Possible Cause	Solution
Poor or no cell attachment upon cell plating	Expired or poor quality of medium due to extended or incorrect storage (i.e. media left at 37ºC or room temperature for too long).	Use medium that is within 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 the table in pg. 6 for recommended coating volumes. ECM coated tissue culturewares should never be dried out. After coating, use immediately or store with coating solution at - 20°C, and use within 6 months.
Poor cell growth	Expired or poor quality of medium due to extended or incorrect storage (i.e. media left at 37ºC or room temperature for too long).	Use medium that is within 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 media is supplemented with growth factors, aliquot into working volumes, date and keep aliquots in recommended storage conditions.
High cell detachment or cell overgrowth during differentiation	Plating density is too high.	Plate cells at 1 x $10^4/cm^2$ when initiating differentiation. This corresponds to 2-2.5 x 10^4 cells per well of a 24-well plate.

	Media changes are too rough and disrupt cell attachment.	Do not use vacuum to aspirate. During media changes, only replace 80% volume. The remaining volume is necessary to buffer against cell perturbation during media changes
	Use of non-recommended tissue culturewares such as 4-well and 8-well chamber slides.	We do not recommend the use of 4-well and 8-well chamber slides. If higher magnification images are required, use 12mm circle glass coverslips that are placed in 24-well plates. Refer to pg. 6-7 for acid-treatment and coating of glass coverslips.
High cell death during differentiation	Low humidity during differentiation.	Fill the outer wells of 24-well plates with sterile distilled water to help maintain 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. 6 for recommended coating volumes.
Poor antibody staining	Inadequate volume of antibody, inaccurate titration or lot to lot variation in antibodies.	If species specificity is unknown, titrations may be necessary to obtain the correct staining dilution. EMD-Millipore's Human Oligodendrocyte Characterization Kit (Catalog # SCR601) is recommended for best results.

FAQs

1. **What tissue culture flask should I thaw the cells out onto?**
   Ans. One vial of cells should be thawed and plated onto an Matrigel coated T25 flask. It is not recommended to thaw to a larger sized flask.

2. **The cells are provided at what passage?**
   Ans. Cells were banked at passage 3 and can be expanded twice. Cells should not be used beyond passage 6.

3. **How many times can I passage the cells?**
   Ans. Cells can be passaged twice. Upon thawing, cells are at passage 4. With an additional two passages, cells are at passage 6. Cells should not be expanded beyond passage 6.

4. **At what confluency should I passage the cells?**
   Ans. Cells should be passaged when they have reached 80% confluency. It is NOT recommended to passage the cells when they are 100% confluent.

5. **What is the optimal splitting ratio?**
   Ans. The optimal seeding density is 2 to 4 x 10^4 / cm^2. This corresponds to 500,000 cells on an appropriately coated T25 flask or 1:3 to 1:5 split.

6. **Can I freeze my cells?**
   Ans. No, we do not recommend freezing the cells. Performance of cells that have been frozen by individual users can not be guaranteed.

7. **Can I use my media instead?**
   Ans. The Human OPC Expansion Media was optimized specifically for EMD Millipore's Human OPCs. 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 Human OPC Expansion or Differentiation Media kits to expand, culture and differentiate oligodendrocytes that I have obtained from another vendor or have isolated myself?**
   Ans. No. These media were optimized specifically for EMD Millipore's Human OPCs. Other cell types have not been tested. We do not believe that cells obtained form other sources or directly isolated from brain tissues will behave similarly in the media system.

9. **Can I use my own growth factors to differentiate?**
   Ans. 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 OPC expansion and differentiation.

10. **Can I use a different ECM to coat?**
    Ans. 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 OPC expansion and differentiation.

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