STEMdiff™ Microglia Differentiation Kit
STEMdiff™ Microglia Maturation Kit

Differentiation and maturation kits for generation of microglia from hPSC-derived hematopoietic progenitor cells

Catalog #100-0019 1 Kit
Catalog #100-0020 1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

The STEMdiff™ microglia culture system comprises STEMdiff™ Microglia Differentiation Kit and STEMdiff™ Microglia Maturation Kit. Together, these kits are used to differentiate and mature microglia derived from human pluripotent stem cells (hPSCs) using STEMdiff™ Hematopoietic Kit (Catalog #05310). The resulting cells are a highly pure population of microglia (> 80% CD45/CD11b-positive, > 50% TREM2-positive microglia; < 20% morphologically distinct monocytes or macrophages). Cells derived using these products are versatile tools for modeling human neurological development and disease, drug screening, toxicity testing, and cell therapy validation.

Product Information

The following components are sold as part of a complete kit (Catalog #100-0019 or 100-0020) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Microglia Differentiation Kit (Catalog #100-0019)				
STEMdiff™ Microglia Basal Medium	100-0021	90 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Microglia Supplement 1	100-0022	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Microglia Supplement 2	100-0023	400 µL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Microglia Maturation Kit (Catalog #100-0020)				
STEMdiff™ Microglia Basal Medium	100-0021	90 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Microglia Supplement 1	100-0022	10 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Microglia Supplement 2	100-0023	400 µL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
STEMdiff™ Microglia Supplement 3	100-0030	400 µL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
DMEM/F-12 with 15 mM HEPES	36254
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
STEMdiff™ Hematopoietic Kit	05310
Trypan Blue	07050

Preparation of Reagents and Materials

For microglia differentiation, coat cultureware with Corning® Matrigel® (section A). For microglia maturation, coat cultureware with Corning® Matrigel®; for immunocytochemistry applications, coat cultureware with poly-D-lysine (PDL) or fibronectin (section B).

A. Coating Cultureware with Corning® Matrigel®

Matrigel® should be aliquoted and frozen. Consult the Matrigel® Certificate of Analysis for the recommended aliquot size ("Dilution Factor") to prepare 25 mL of diluted matrix. Ensure to always keep Matrigel® on ice when thawing and handling to prevent it from gelling.

NOTE: Use tissue culture-treated cultureware.

1. Thaw one aliquot of Matrigel® on ice.
2. Dispense 25 mL of cold DMEM/F-12 with 15 mM HEPES into a 50 mL conical tube and keep on ice.
3. Add thawed Matrigel® to the cold DMEM/F-12 with 15 mM HEPES (in the 50 mL tube) and mix well. The vial may be washed with cold medium if desired.
4. Immediately use the diluted Matrigel® solution to coat tissue culture-treated cultureware. See Table 1 for recommended coating volumes.
5. Swirl the cultureware to spread the solution evenly across the surface.

   NOTE: If the surface of the cultureware is not fully coated by the Matrigel® solution, it should not be used.
6. Incubate at room temperature (15 - 25°C) for at least 1 hour before use. Do not let the Matrigel® solution evaporate.

   NOTE: If not used immediately, seal the cultureware with Parafilm® to prevent evaporation of the Matrigel® solution; store at 2 - 8°C for up to 1 week after coating. Allow stored coated cultureware to come to room temperature for 30 minutes before proceeding to step 7.
7. Immediately prior to seeding cells, gently tilt the cultureware onto one side and allow the excess solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.

Table 1: Recommended Volumes for Coating Cultureware

CULTUREWARE	APPROXIMATE SURFACE AREA	VOLUME OF COATING SOLUTION
96-well plate	0.32 cm^2/well	50 µL/well
24-well plate	1.9 cm^2/well	250 µL/well
12-well plate	3.8 cm^2/well	500 µL/well
6-well plate	9.5 cm^2/well	1 mL/well
35 mm dish	9 cm^2	1 mL
60 mm dish	21 cm^2	2.5 mL

B. Coating Cultureware with Poly-D-Lysine (PDL) or Fibronectin

1. Prepare a PDL or fibronectin stock solution as follows:

   ### 10 µg/mL PDL Stock Solution

   - Dissolve 5 mg PDL (e.g. Sigma Catalog #P7280) in 50 mL sterile water to prepare a 100 µg/mL solution. Store in a polypropylene vial at 2 - 8°C for up to 3 months.
   - Dilute the 100 µg/mL PDL solution 1 in 10 with sterile water to a final concentration of 10 µg/mL.

   OR

   ### 1 µg/mL Fibronectin Stock Solution

   - Dilute Fibronectin (1 mg/mL; Catalog #07159) 1 in 1000 with DMEM/F-12 with 15 mM HEPES. Store at 2 - 8°C for up to 1 week.

     NOTE: Fibronectin is not stable at room temperature (15 - 25°C); avoid vortexing or excessive agitation.
2. Coat tissue culture-treated cultureware with PDL or fibronectin (prepared in step 1); see Table 1 for recommended coating volumes.
3. Swirl the cultureware to spread the solution evenly across the surface.
4. Incubate at room temperature for 3 hours or at 2 - 8°C overnight (~20 hours). Seal cultureware to prevent evaporation. Coated cultureware can be stored at 2 - 8°C for up to 1 week after coating.
5. Immediately prior to seeding cells, gently tilt the cultureware onto one side and allow the excess solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.

   NOTE: If PDL was used for coating, wash each well with sterile phosphate-buffered saline (PBS) or DMEM/F-12 with 15 mM HEPES prior to use.

C. Preparation of STEMdiff™ Microglia Differentiation Medium

Use sterile technique to prepare STEMdiff™ Microglia Differentiation Medium (Basal Medium + Supplement 1 + Supplement 2). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw Supplements 1 & 2 at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.

   NOTE: If not used immediately, aliquot supplements and store at -20°C. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 10 mL of Supplement 1 and 400 µL of Supplement 2 to 90 mL of Basal Medium. Mix thoroughly.

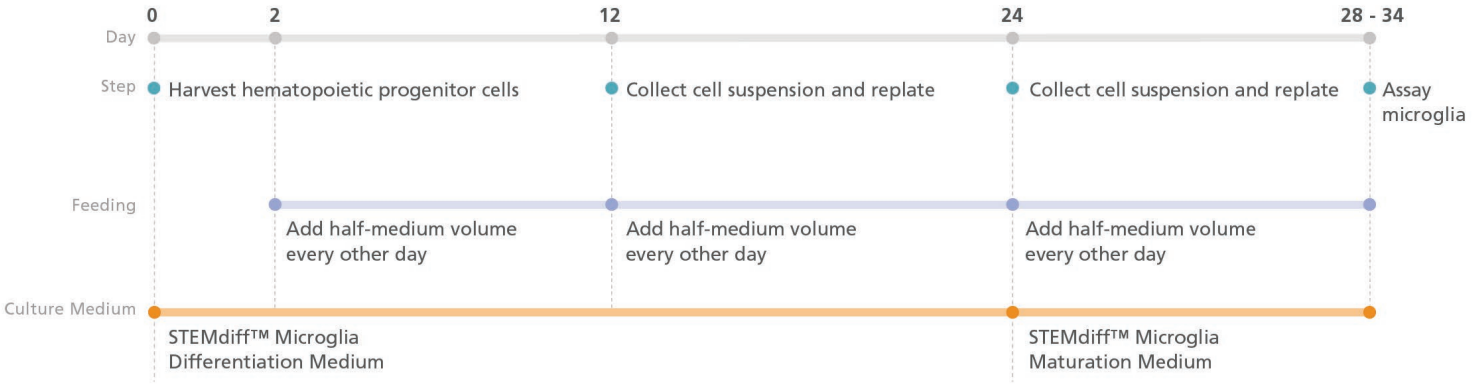
   NOTE: If not used immediately, store STEMdiff™ Microglia Differentiation Medium at 2 - 8°C for up to 4 weeks. Warm medium to 37°C before use.

D. Preparation of STEMdiff™ Microglia Maturation Medium

Use sterile technique to prepare STEMdiff™ Microglia Maturation Medium (Basal Medium + Supplements 1, 2, & 3). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw Supplements 1, 2, & 3 at room temperature (15 - 25°C) or at 2 - 8°C overnight. Mix thoroughly.
   NOTE: If not used immediately, aliquot supplements and store at -20°C. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 10 mL of Supplement 1, 400 µL of Supplement 2, and 400 µL of Supplement 3 to 90 mL of Basal Medium. Mix thoroughly.
   NOTE: If not used immediately, store STEMdiff™ Microglia Maturation Medium at 2 - 8°C for up to 4 weeks. Warm medium to 37°C before use.

Protocol Diagram



Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols.

A. Generation of Hematopoietic Progenitor Cells

Generate hematopoietic progenitor cells (HPCs) from hPSCs using STEMdiff™ Hematopoietic Kit (Catalog #05310). For complete instructions, refer to the Product Information Sheet (Document #10000003456), available at www.stemcell.com, or contact us to request a copy.

NOTE: Ensure HPCs are ≥ 90% CD43+ before proceeding to section B. CD43 expression may be measured by flow cytometry after labeling with Anti-Human CD43 Antibody, Clone CD43-10G7 (Catalog #60085).

B. Microglia Differentiation

The following instructions are for a single well of a 6-well plate. If using other cultureware, adjust volumes accordingly.

1. Day 0: Harvest suspended hematopoietic progenitor cells (day 12 of the STEMdiff™ Hematopoietic Kit protocol). Count cells using Trypan Blue and a hemocytometer.
2. Add 1 x 10^5 - 2 x 10^5 cells to one well of a Matrigel®-coated 6-well plate containing 2 mL STEMdiff™ Microglia Differentiation Medium (cell density 1.1 x 10^4 - 2.2 x 10^4 cells/cm^2). Incubate at 37°C and 5% CO_2.
3. Feed the cells every other day by topping up the well with half of the start volume of STEMdiff™ Microglia Differentiation Medium (i.e. 1 mL). Do not remove existing medium.
4. Day 12: Transfer the entire cell suspension to a 15 mL conical tube.
   NOTE: The cell population will be semi-adherent when cultured on Matrigel®-coated plates. Some clumping of the cells is normal. Some cells may be loosely attached to the plate, while others may remain entirely in suspension. To collect any remaining loosely adherent cells, the wells may be rinsed using 1 mL of room temperature DMEM/F-12 with 15 mM HEPES after transferring the cell suspension. This wash volume may be pooled with the initial collected cell suspension.
5. Centrifuge the collected cell suspension at 300 x g for 5 minutes.
6. Remove supernatant until there is ~1 mL remaining on top of the cell pellet. Using a pipettor, gently mix to resuspend.
7. Add the cell suspension to one well of a new Matrigel®-coated 6-well plate containing 1 mL fresh STEMdiff™ Microglia Differentiation Medium. Incubate at 37°C and 5% CO_2.
8. Feed the cells every other day for 12 days by topping up the well with half of the start volume of STEMdiff™ Microglia Differentiation Medium (i.e. 1 mL).
9. Day 24: Proceed to section C for microglia maturation.

C. Microglia Maturation

When cells have been cultured for 24 days in STEMdiff™ Microglia Differentiation Medium, prepare STEMdiff™ Microglia Maturation Medium and collect the cells as described below.

The following instructions are for a single well of a 6-well plate. For other cultureware, adjust volumes accordingly.

1. Day 24: Transfer the entire cell suspension to a 15 mL conical tube.

   NOTE: The cell population will be semi-adherent when cultured on Matrigel®-coated plates. Some clumping of the cells is normal. Some cells may be loosely attached to the plate, while others may remain entirely in suspension. To collect any remaining loosely adherent cells, the wells may be rinsed using 1 mL of room temperature DMEM/F-12 with 15 mM HEPES after transferring the cell suspension. This wash volume may be pooled with the initial collected cell suspension.
2. Centrifuge the collected cell suspension at 300 x g for 5 minutes.
3. Remove supernatant until there is ~1 mL remaining on top of the cell pellet. Using a pipettor, gently mix to resuspend.
4. Add the cell suspension to one well of a new Matrigel®-coated 6-well plate containing 1 mL fresh STEMdiff™ Microglia Maturation Medium. Incubate at 37°C and 5% CO_2.

   NOTE: If a specific seeding density is required, count the cells using Trypan Blue and a hemocytometer, and replate cells at a minimum of 1.1 x 10^4 cells/cm^2.

   NOTE: If cells are to be used for immunocytochemistry, use cultureware coated with either 10 µg/mL PDL or 1 µg/mL fibronectin (see Preparation of Reagents and Materials section), which will promote stronger attachment of microglia to the plate surface. For other applications, use Matrigel®-coated cultureware.
5. Feed the cells every other day by topping up the well with half of the start volume of STEMdiff™ Microglia Maturation Medium (i.e. 1 mL).
6. Day 28 - 34: Microglia are terminally differentiated after 4 - 10 days in STEMdiff™ Microglia Maturation Medium. There will be limited proliferation of cells after Day 28. After 10 days in maturation medium, there may be an increase in cell death.

Assessment of Microglia Differentiation

For evaluating microglia differentiation efficiency, marker expression may be assessed by flow cytometry as early as Day 24. Refer to Table 2 below for recommended antibody clones and expected expression levels upon a successful differentiation experiment. Results may vary depending on the cell line used.

Table 2. Recommended Antibody Clones and Expected Expression Levels for Microglia Assessment by Flow Cytometry

ANTIBODY TARGET	SUGGESTED CLONE	EXPECTED EXPRESSION (% BY FLOW CYTOMETRY)
CD45	HI30 (Catalog #60018)	> 80% co-expression of CD45 and CD11b
CD11b	ICRF44 (Catalog #60040)	
TREM2	237920 (R&D Systems, FAB17291S)	> 50%

The resulting cells may also be characterized by immunocytochemistry for microglia markers or by May-Grunwald Giemsa staining. Contact techsupport@stemcell.com for more information.

Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com, or contact us at techsupport@stemcell.com.