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

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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 #
STEMdiff™ Hematopoietic Kit	05310
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
DMEM/F-12 with 15 mM HEPES	36254
Conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
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®, or 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	0.33 cm^2/well	50 µL/well
4- or 24-well plate	2 cm^2/well	250 µL/well
6-well plate	10 cm^2/well	1 mL/well
12-well plate	3.5 cm^2/well	500 µL/well
35 mm dish	10 cm^2	1.5 mL
60 mm dish	20 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.

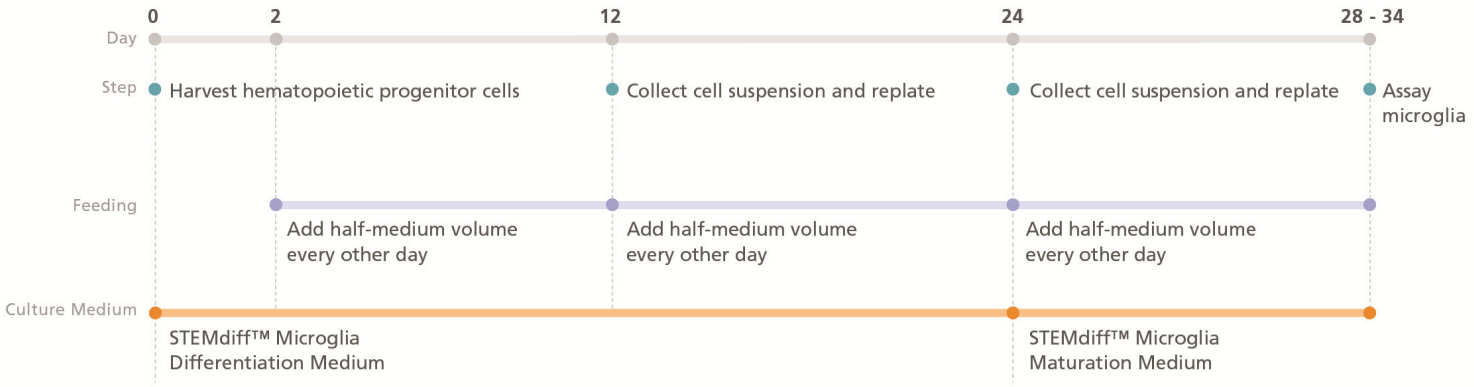
   NOTE: If not used immediately, store STEMdiff™ Microglia Differentiation Medium at 2 - 8°C for up to 4 weeks. Warm medium to 37°C before use.

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. Microglia Differentiation

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

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. Centrifuge at 300 x *g* for 5 minutes.
5. Remove supernatant until there is ~1 mL remaining on top of the cell pellet. Using a pipettor, gently mix to resuspend.
6. 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.
7. 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).
8. Day 24: Proceed to section B for microglia maturation.

B. 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. Centrifuge at 300 x *g* for 5 minutes.
2. Remove supernatant until there is ~1 mL remaining on top of the cell pellet. Using a pipettor, gently mix to resuspend. Count cells using Trypan Blue and a hemocytometer.
3. Add 1 x 10^6 cells to one well of a new coated 6-well plate containing 1 mL fresh STEMdiff™ Microglia Maturation Medium (cell density 1 x 10^5 cells/cm^2). If the total volume in the well is < 2 mL, top up to 2 mL with Maturation Medium. Incubate at 37°C and 5% CO_2.

   NOTE: 1 x 10^5 cells/cm^2 is the optimal density for replating. Continuing the protocol with < 5 x 10^5 cells (5.5 x 10^4 cells/cm^2) may result in a decrease in the % CD11b-positive cells at the end of maturation.

   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 section). For other applications, use Matrigel®-coated cultureware.
4. 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).
5. Day 28 - 34: Microglia are mature after 4 - 10 days in STEMdiff™ Microglia Maturation Medium. These cells have limited capacity for expansion; after 10 days of culture there may be an increase in cell death.

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.