Human iPSC-Derived Microglia

Frozen microglia differentiated from human induced pluripotent stem cell line, SCTi003-A

Catalog #200-1100	1 x 10^6 cells
#200-1101	5 x 10^6 cells

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Product Description

Human induced pluripotent stem cell (iPSC)-Derived Microglia are manufactured from Healthy Control Human iPSC Line, Female, SCTi003-A (Catalog #200-0511), using STEMdiff™ Hematopoietic Kit (Catalog #05310) to generate hematopoietic progenitor cells, followed by an in-house protocol optimized for scalable and consistent manufacturing of highly pure, functional human microglia.

Human iPSC-Derived Microglia should be thawed and further cultured using STEMdiff™ Microglia Maturation Kit (Catalog #100-0020), resulting in a population of microglia with >70% co-expression of CD45 and CD11b and >70% expression of P2RY12 and TREM2. These microglia are functional and non-activated, and can be maintained for up to 20 days post-thaw. Human iPSC-Derived Microglia are versatile tools for modeling human neurological development and diseases, drug discovery, and toxicity testing.

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Stability and Storage

Cells are frozen in a cryopreservation medium containing dimethyl sulfoxide (DMSO). The product is stable at -135°C or colder for 12 months from date of receipt. Thawed samples must be used immediately.

Precautions

Cell Screening: iPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, Herpes Virus Type 7, Herpes Virus Type 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS.

NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Bovine Serum Albumin (BSA), cell culture grade	e.g. Gibco 15260037
Conical tubes, 15 mL and 50 mL	e.g.100-0092 and 100-0090
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
DMEM/F-12 with 15 mM HEPES	36254
Fibronectin	07159
Gentle Cell Dissociation Reagent	100-0485
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
Poly-D-Lysine	e.g. Sigma P7280
STEMdiff™ Microglia Maturation Kit	100-0020
Trypan Blue	07050
Y-27632 (Dihydrochloride)	72302

Preparation of Reagents and Materials

For microglia maintenance and applications other than immunocytochemistry assays, coat cultureware with Corning® Matrigel® hESC-Qualified Matrix (Corning® Matrigel®; see section A). For immunocytochemistry assays, coat the cultureware with poly-D-lysine (PDL) or fibronectin (see section B) which will promote stronger attachment of microglia to the plate surface.

A. COATING CULTUREWARE WITH CORNING® MATRIGEL®

Using sterile technique, coat cultureware with Corning® Matrigel® prior to initial plating of Human iPSC-Derived Microglia. Corning® 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 Corning® Matrigel® on ice when thawing and handling to prevent it from gelling.

NOTE: Use tissue culture-treated cultureware.

1. Thaw one aliquot of Corning® 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 Corning® Matrigel® to the cold DMEM/F-12 with 15 mM HEPES (in the 50 mL conical tube) and mix thoroughly. If desired, wash the vial with cold medium.
4. Immediately use the diluted Corning® Matrigel® solution to coat tissue culture-treated cultureware. Refer to Table 1 for recommended coating volumes.
5. Swirl the cultureware to spread the diluted Corning® Matrigel® solution evenly across the surface.

   NOTE: If the surface of the cultureware is not fully coated by the 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 diluted Corning® Matrigel® solution evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the diluted Corning® Matrigel® solution (e.g. with Parafilm®) and can be stored at 2 - 8°C for up to 1 week after coating. Allow stored coated cultureware to come to room temperature for 30 minutes before continuing to step 7.
7. Immediately prior to seeding cells, gently tilt the cultureware onto one side and allow the excess Corning® Matrigel® 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 and Plating Various Cultureware

CULTUREWARE	APPROXIMATE SURFACE AREA	VOLUME OF COATING SOLUTION	VOLUME OF MEDIUM
96-well plate	0.33 cm^2/well	50 µL/well	100 µL/well
24- or 48-well plate	2 cm^2/well	250 µL/well	500 µL/well
12-well plate	4 cm^2/well	500 µL/well	1 mL/well
6-well plate	10 cm^2/well	1 mL/well	2 mL/well
35 mm dish	10 cm^2	1 mL	2 mL
60 mm dish	20 cm^2	2.5 mL	5 mL

B. COATING CULTUREWARE WITH PDL OR FIBRONECTIN

1. Prepare a PDL or fibronectin solution as follows:

   PDL Solution (10 µg/mL)

   a. Dissolve 5 mg PDL 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.

   b. Dilute the 100 µg/mL PDL solution 1 in 10 with sterile water to a final concentration of 10 µg/mL.

   Fibronectin Solution (1 µg/mL)

   Dilute Fibronectin (1 mg/mL) 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 volumes of coating solution.
3. Swirl the cultureware to spread the solution evenly across the surface.
4. Incubate at room temperature (15 - 25°C) 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 thoroughly with sterile phosphate-buffered saline (PBS) or DMEM/F-12 with 15 mM HEPES prior to use.

C. COMPLETE STEMdiff™ MICROGLIA MATURATION MEDIUM

Use sterile technique to prepare complete STEMdiff™ Microglia Maturation Medium (STEMdiff™ Microglia Basal Medium + STEMdiff™ Microglia Supplements 1, 2, & 3).

For complete instructions on preparing complete STEMdiff™ Microglia Maturation Medium, refer to Product Information Sheet (PIS; Document #10000006003) available at www.stemcell.com, or contact us to request a copy.

- For thawing and plating Human iPSC-Derived Microglia, add Y-27632 (Dihydrochloride) to complete STEMdiff™ Microglia Maturation Medium as follows:
  1. Reconstitute Y-27632 (Dihydrochloride) to a final concentration of 5 mM with sterile water. If not used immediately, aliquot and store at -20°C for up to 6 months.
  2. Add Y-27632 (Dihydrochloride) to complete STEMdiff™ Microglia Maturation Medium for a final concentration of 10 µM.
- For feeding and replating Human iPSC-Derived Microglia, use complete STEMdiff™ Microglia Maturation Medium.

Directions for Use

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

IMPORTANT: To confirm the number of cells provided, a viable cell count must be done immediately after thawing (before washing). Work quickly once the cells have been thawed to ensure high viability and recovery. Use sterile technique when processing thawed cells.

A. THAWING AND PLATING HUMAN iPSC-DERIVED MICROGLIA

1. Use tissue culture-treated cultureware and coat your cultureware according to the application (see Preparation of Reagents and Materials, sections A & B).

2. Warm DMEM/F-12 with 15 mM HEPES, and complete STEMdiff™ Microglia Maturation Medium with 10 µM Y-27632 (Dihydrochloride; see Preparation of Reagents and Materials, section C) to 37°C before starting the protocol to ensure that the thawing procedure is done as quickly as possible.
3. Add 10 mL of warm DMEM/F-12 with 15 mM HEPES to a 15 mL conical tube.

   NOTE: To improve recovery, add 1% BSA to DMEM/F-12 with 15 mM HEPES.
4. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
5. In a biosafety hood, twist the cap a quarter-turn to relieve internal pressure and then retighten.
6. Quickly thaw cells in a 37°C water bath by gently shaking the cryovial continuously until only a small frozen cell pellet remains. Do not vortex cells.

   NOTE: ThawSTAR® CFT2 Automated Thawing System (Catalog #100-0650) may be used to quickly and efficiently thaw cells. For complete instructions, refer to the PIS (Document #10000010334), available at www.stemcell.com, or contact us to request a copy.
7. Remove the cryovial from the water bath and wipe it with 70% ethanol or isopropanol.

   NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery.
8. In a biosafety hood, measure and record the total volume of the cell suspension using a 2 mL serological pipette.
9. Remove a 20 µL aliquot of cells for pre-wash counting. If using Trypan Blue to assess viability, dilute with a minimum of 20 µL of medium and record the volume of medium added. Count cells using a hemocytometer.
10. Transfer cells from the cryovial to the tube containing 10 mL of warm DMEM/F-12 with 15 mM HEPES. Mix gently.
11. Rinse the vial with 1 mL of warm DMEM/F-12 with 15 mM HEPES and add it dropwise to the cells, while gently swirling the 15 mL tube.
12. Centrifuge cells at 300 x g for 5 minutes at room temperature (15 - 25°C).
13. Aspirate medium, leaving the cell pellet intact.
14. Gently resuspend the cell pellet in 1 mL of complete STEMdiff™ Microglia Maturation Medium with 10 µM Y-27632 (Dihydrochloride).
15. Remove a 20 µL aliquot of cells for post-wash counting. If using Trypan Blue to assess viability, dilute with a minimum of 20 µL of medium and record the volume of medium added. Count cells using a hemocytometer. Use the final post-wash cell concentration to calculate the volume of cell suspension to plate per well in step 17.
16. Using a serological pipette or by aspiration, gently remove the excess coating solution from the coated cultureware (prepared in step 1). Ensure that the coated surface is not scratched.
17. Seed cells onto a coated cultureware at a minimum density of 1.1 x 10^4 cells/cm^2 in complete STEMdiff™ Microglia Maturation Medium with 10 µM Y-27632 (Dihydrochloride). Refer to Table 1 for recommended volumes.

    NOTE: The seeding density of Human iPSC-Derived Microglia should be optimized for the application. For example, seed 1 x 10^5 cells per well of a 24-well plate for lipopolysaccharide-induced activation experiments. Expected post-thaw viability is typically 70 - 90%.
18. Place the plate in a 37°C and 5% CO_2 incubator.
19. Feed cells every other day by topping up the well with a **half-volume** of complete STEMdiff™ Microglia Maturation Medium (i.e. 1 mL/well for a 6-well plate) without removing the existing medium. On Day 12, the well will reach the maximum volume, and the cell suspension will need to be replated (See Directions for Use, section B).

    NOTE: It is recommended to allow microglia to recover for at least 1 day before using in downstream assays. Confirm that the thawing process was successful with a low amount of cell death. If there is a significant amount of cell death or clumping, replate the microglia after 3 - 7 days of culture before using in downstream assays. Contact techsupport@stemcell.com for more information.

B. REPLATING HUMAN iPSC-DERIVED MICROGLIA

The following protocol is for replating Human iPSC-Derived Microglia in a 6-well plate. If using other cultureware, adjust volumes accordingly.

1. Use tissue culture-treated cultureware and coat your cultureware according to the application (see Preparation of Reagents and Materials, sections A & B).
2. Warm DMEM/F-12 with 15 mM HEPES and complete STEMdiff™ Microglia Maturation Medium (see Preparation of Reagents and Materials, section C) to 37°C.
3. Transfer the entire cell suspension to a 15 mL conical tube.

   NOTE: The cell population will be semi-adherent when cultured on coated cultureware plates and some cell clumping is normal. To collect the cells still loosely attached to the plate, the wells may be incubated with 1 mL of Gentle Cell Dissociation Reagent for 5 minutes at room temperature (15 - 25°C) and rinsed using 1 mL of DMEM/F-12 with 15 mM HEPES. These wash volumes should be pooled with the initial collected cell suspension.
4. Centrifuge cells at 300 x g for 5 minutes at room temperature.
5. Aspirate medium, leaving the cell pellet intact.
6. Gently resuspend the cell pellet in 1 mL of complete STEMdiff™ Microglia Maturation Medium.

7. Remove a 20 µL aliquot of cells for counting. If using Trypan Blue to assess viability, dilute with a minimum of 20 µL of medium and record the volume of medium added. Count cells using a hemocytometer. Use the final post-wash cell concentration to calculate the volume of cell suspension to plate per well in step 9.
8. Using a serological pipette or by aspiration, gently remove the excess coating solution from the coated cultureware (prepared in step 1). Ensure that the coated surface is not scratched.
9. Seed cells onto coated cultureware at a minimum density of 1.1 x 10^4 cells/cm^2 in complete STEMdiff™ Microglia Maturation Medium. Refer to Table 1 for recommended volumes.

   NOTE: The seeding density of Human iPSC-Derived Microglia should be optimized for each application.
10. Place the plate in a 37°C and 5% CO_2 incubator.
11. Feed cells every other day by topping up the well with a half-volume of complete STEMdiff™ Microglia Maturation Medium (i.e. 1 mL/well for a 6-well plate) without removing the existing medium.

Assessment of Microglia

For evaluating microglia, marker expression may be assessed by flow cytometry. Refer to Table 2 for recommended antibody clones and expected expression levels upon a successful thaw.

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)	> 70% co-expression of CD45 and CD11b
CD11b	ICRF44 (Catalog #60040) or M1/70 (Catalog #60001)	
P2RY12	S16001E (Biolegend, 392103)	> 70%
TREM2	237920R (R&D Systems, FAB17291RN)	

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.