Applied StemCell, Inc.
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Datasheet

iPSC-Derived Human Microglia Cells (iMGLs) Starter Kit

Product Information

Catalog Number **ASE-9601**

Description

Microglia are the primary immune cells in the central nervous system (CNS) and they play a crucial role in maintaining neuronal homeostasis and synaptic plasticity for normal brain development and neuronal function, and inflammatory responses. They have also been implicated in the pathogenesis of several neurological disorders such as Alzheimer's disease and Parkinson's disease. The differentiation of iPSCs to microglia provide a steady source of primary microglia-like cells without the sourcing issues associated with primary microglia. These cells are an excellent physiologically relevant research model to study immune response mechanisms in the brain, neuronal function, and for disease modeling.

Applied StemCell has developed an efficient cytokine-based method to differentiate high-quality microglia cells from human iPSCs (iMGLs), which recapitulate the phenotype and functional parameters of primary microglia and *in vivo* microglial cells. Our proprietary erythromyeloid induction protocol mimics the *in vivo* activation pathway for the development of microglia in the brain from hematopoietic stem cells.

The iMGLs were derived through directed differentiation from an integration-free, control human iPSC line (ASE-9211). These high-purity (≥90%) cells express microglial-specific markers TMEM119, and P2Ry12+ (also referred to as P2Y12+) as well as IBA1, CX3CR1+, and TREM2+ (Figure 1). The cells are provided as fully-differentiated, cryopreserved cells that you can readily recover and culture for your experiments. The iMGLs are functionally viable for 7 days after recovery.

To harness the full potential of our iMGLs, we also provide optimized, serum-free Microglia Culture Media (ASE-9601MM) that supports robust maintenance and functionality of the iMGLs in culture.

Parental Tissue

Control human iPSC (ASE-9211); p15
Age: Neonate
Gender: Male
Ethnicity: African-American
Tissue Source: Dermal Fibroblasts
Reprogramming Method: Episomal
Culture Conditions: Feeder-free

Clinical information

Healthy (with no known disease phenotypes)

Shipping

Dry ice

Storage and Stability

Store the components of the kit at the appropriate storage conditions as indicated in the media and materials table, immediately upon arrival. Shelf-life of the product is contingent upon proper storage conditions

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Quality Control

Each lot of iPSC-derived human microglia cells has been tested for growth, viability and purity (≥90%) following recovery from cryopreservation. In addition, each lot has been tested for expression of microglial markers (TMEM119 and P2RY12), and for the absence of mycoplasma and pathogens.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling frozen vials. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Warranty

Performance of Applied StemCell's iMGLs has been validated with the microglia culture media of the iMGL Starter Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the iMGL Starter Kit and those recommended are used to culture ASE-9601 microglial precursors.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Characterization of the iMGL

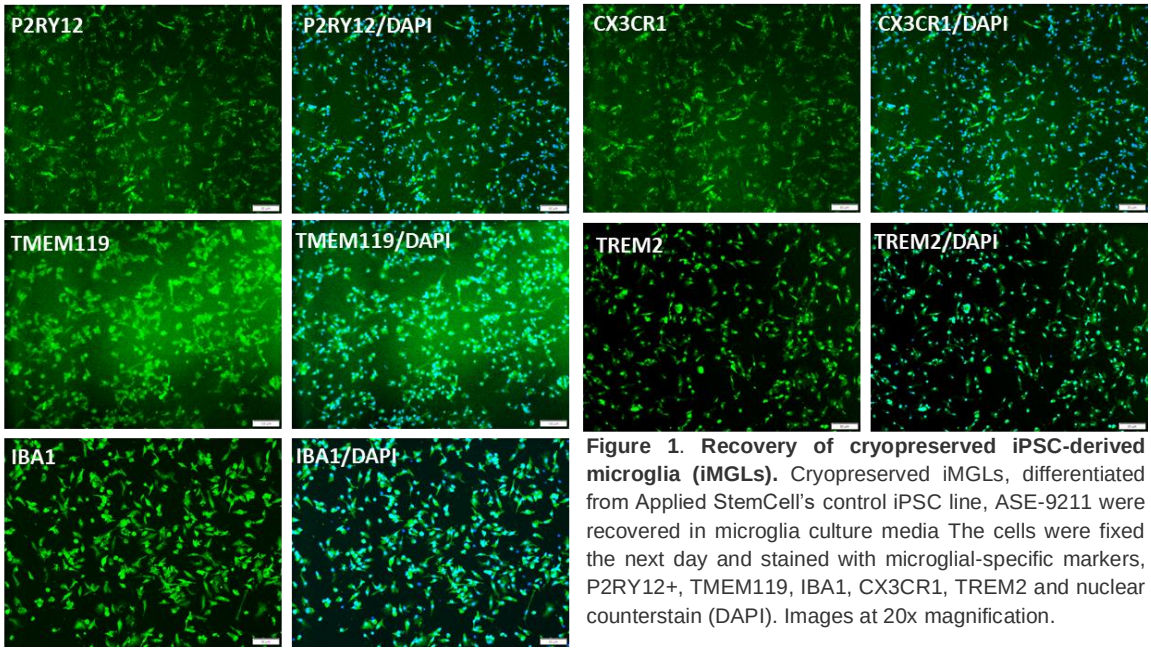

Figure 1. Recovery of cryopreserved iPSC-derived microglia (iMGLs). Cryopreserved iMGLs, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in microglia culture media The cells were fixed the next day and stained with microglial-specific markers, P2RY12+, TMEM119, IBA1, CX3CR1, TREM2 and nuclear counterstain (DAPI). Images at 20x magnification.

Media and Material

Microglia Starter Kit (ASE-9601)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9601-C	iPSC-derived Microglia (iMGLs)	≥1 x 10^6 cells/ vial	Liq. N2	12 months
ASE-9601MM	Microglia Basal Culture Media	100 mL	-20°C	12 months
ASE-9601MM-A	Microglia Culture Media Supplement A (20x)	3 mL	-20°C	12 months

Additional Reagents Required

The below reagents are recommended for use with the iMGLs. If you use reagents other than those recommended, we suggest that you do a batch-test to validate integrity of the cells and culture protocol.

- Poly-D-lysine, Sigma, Cat# P7280
- Primary antibodies:
  - Anti-Iba1 Antibody, Abcam, Cat# ab8004
  - Recombinant Anti-TMEM119 Antibody [28-3] - Microglial Marker, Abcam, Cat# 209064
  - Purified anti-P2RY12 Antibody, Biolegend, Cat# S16007D
  - Anti-CX3CR1 Antibody, Abcam, Cat# ab8020
  - Anti-TREM2 Antibody, Abcam, Cat# ab95470
- Secondary antibodies: corresponding secondary antibodies were purchased from ThermoFisher
- BAMBANKER® Serum-Free Cell Freezing Medium, Fujifilm, Cat# 302-14681

Protocol

1. Coating Cell Culture Vessels with Coating Matrix

1.1 Coat the plates with 10µg/mL poly-L-lysine or poly-D-lysine
Note: Please follow manufacturer's instructions in coating plates using poly-L-lysine or poly-D-lysine.

1.2 Incubate at room temperature for at least 1 hour before use
Note: The plates do not need to be washed or dried before seeding the cells.

2. Preparation of Microglia Culture Media

2.1 Thaw the microglia basal culture media and supplement at room temperature before thawing the cryopreserved iMGLs.
2.2 Mix 95 mL of the basal culture media and 5 mL of the supplement to make the microglia complete media.
Note: [Optional] add 1mL penicillin/streptomycin to 100 mL of the complete media to prevent bacterial contamination.

2.3 The complete media should be aliquoted and stored at -20°C if it will not be used immediately.
2.4 The complete media can be stored at 4°C for up to 2 weeks or at -20°C for up to 6 months.

3. Thawing and Culturing Cryopreserved iMGLs

3.1 To thaw the cryopreserved iMGLs, remove one vial from the storage unit.
3.2 Immerse the vial in the bath (up to 2/3rd of the vial) and thaw the cells rapidly until only a small piece of ice is still visible (approximately one minute).
Note: Do not shake the vial during thawing.

3.3 Bring the vial to the biological cabinet immediately and spray the outside of the vial thoroughly with 70% ethanol and wipe it with an autoclaved paper towel.
3.4 Remove the cells from the vial using a p1000 micropipette (or serological pipette) and transfer it slowly, drop-wise while swirling into a 15 mL conical tube containing 5 mL of pre-warmed microglia complete culture medium. Wash the vial with 1 mL medium from the 15 mL conical tube and transfer it back to the tube.
Note: Do not mix cells up and down and avoid generation of bubbles.

3.5 Centrifuge cells at 300 x g for 5 minutes at room temperature.
3.6 Aspirate the medium very carefully using a vacuum (or pipette if preferred), leaving only a drop of liquid in the tube.
Note: Take extra care not to remove or disturb the cell pellet during aspiration of medium.

3.7 Using a p1000 micropipette, add 1 mL of the pre-warmed microglia culture medium into the tube and gently re-suspend cells by pipetting up and down 2-3 times.
3.8 Remove a 10 µL aliquot of the cell suspension and mix it with 10 µL of Trypan blue solution.
3.9 Count the cells.
3.10 Aspirate the coating matrix from the pre-warmed cell culture vessel.
3.11 Seed the microglial cells at a density ranging from 5,000 – 10,000 live cells/cm2. Distribute the cells evenly.
3.12 Place cell culture vessels in the incubator (37°C/ 5% CO2/ humidity control) overnight.
3.13 A half media change is recommended for every 3 days.
Note: there are always some live cells keep floating up from the plates. You can transfer the floating cells to another pre-coated plates and they will re-attach the next day.

3.14 We recommend using the recovered iMGLs within 1-2 weeks after recovery.
3.15 Treat the cells with pre-chilled media to detach the cells for re-seeding or cryopreservation purpose.

4. Subculture and Cryopreservation of iMGLs

4.1. Add 2 mL per well (of a 6-well plate) pre-chilled microglia complete media to the cells
4.2. Detach the cells by tapping the plates until most of the cells detach from the plates.
4.3. Transfer the detached cells to a conical tube using a p1000 micropipette.
4.4. Centrifuge the cells at 300 x g for 5 minutes.
4.5. Aspirate the supernatant without disturbing the cell pellet.
4.6. Resuspend the cells with 2 mL fresh pre-warmed complete media for sub-culture or 1 mL of BAMBANKER® Serum Free Cell Freezing Medium for cryopreservation.