Human Microglia Cells (derived from Normal, Male, iPSC line)

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 ASE-9601A iMGLs were derived through directed differentiation from an integration-free, control human iPSC line reprogrammed from fibroblasts of a Caucasian male donor. These high-purity (≥90%) cells express microglial-specific markers TMEM119, and P2Ry12+ (also referred to as P2Y12+) as well as IBA1, CX3CR1+, and TREM2+. 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.

Catalog #	Product	Size
ASE-9601A	Human iMGLs (from Normal, Male, iPSCs	≥ 1.0×10^6 cells/vial

Parental Tissue Information	
Parental Cell Line	Control Human iPSCs
Tissue Source	Dermal Fibroblast
Sex	Male
Age	39
Race	Caucasian
Clinical Information	Normal

Intended Use

The ASE-9601A iMGL cell line is an excellent physiologically relevant research model to study immune response mechanisms in the brain, neuronal function, and for disease modeling.

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

Quality Assurance

Each lot of ASE-9610A iMGL cells has been tested for viability and purity (≥90%) following recovery from cryopreservation, expression of microglial markers (TMEM119 and P2RY12) and for normal male karyotype. Additionally, these iPSCs have been tested to confirm the absence of bacterial, fungal, and mycoplasma contamination.

All quality control test results are reported on a lot-specific Certificate of Analysis, available at appliedstemcell.com or upon request.

Storage Instructions 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.

Precautions

The safety and efficacy of this product in diagnostic or other clinical uses have not been established. This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials while they are submerged in a liquid nitrogen freezer. Durning rapid 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. When retrieving the cell vial from a liquid nitrogen freezer, carefully twist open the cap to release any built-up nitrogen gas prior to thawing the cells.

Refer to the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Directions for Use

Kit Components

Catalog#	Component	Amount	Storage	Shelf Life
ASE-9601A-C	iMGLs	≥ 1.0×10^6 cells/vial	Liq. N_2	12 months
ASE-9601MM	Microglia Basal Medium	20 mL	-20°C	12 months
ASE-9601MM-A	Microglia Medium Supplement (20x)	1 mL	-20°C	12 months

Required Materials (Not Provided)

The reagents below are recommended for use with the iMGLs. If you use reagents other than those recommended, we suggest that you make a test batch to validate integrity of the cells and culture protocol. Primary antibodies are listed in the table, and the corresponding secondary antibodies were purchased from ThermoFisher.

Product	Vendor	Catalog Num.
Poly-D-lysine	Sigma	P7280
Ani-Iba1 Antibody	Abcam	ab8004
Recombinant Anti-TMEM119 Antibody [28-3] – Microglial Marker	Abcam	209064
Purified anti-P2RY12 Antibody	Biolegend	S16007D
Anti-CX3CR1 Antibody	Abcam	ab8020
Anti-TREM2 Antibody	Abcam	ab95470
BAMBANKER® Serum-Free Cell Freezing Medium	Fujifilm	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-L-lysine or poly-D-lysine.

    Notes: Please follow the manufacturer's instructions when 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 19 mL of the basal culture media and 1 mL of the supplement to make the microglia complete media.

    Note: [Optional] add 0.2mL penicillin/streptomycin to 20 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 50,000 - 100,000 live cells/cm^2. Distribute the cells evenly.
3.12. Place cell culture vessels in the incubator (37°C/ 5% CO_2/ humidity control) overnight.
3.13. A half media change is recommended every 3 days.

Note: There are always some live cells that float up from the plates. You can transfer the floating cells to another pre-coated plate 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.

Data

Characterization of ASE-9601A iMGLs

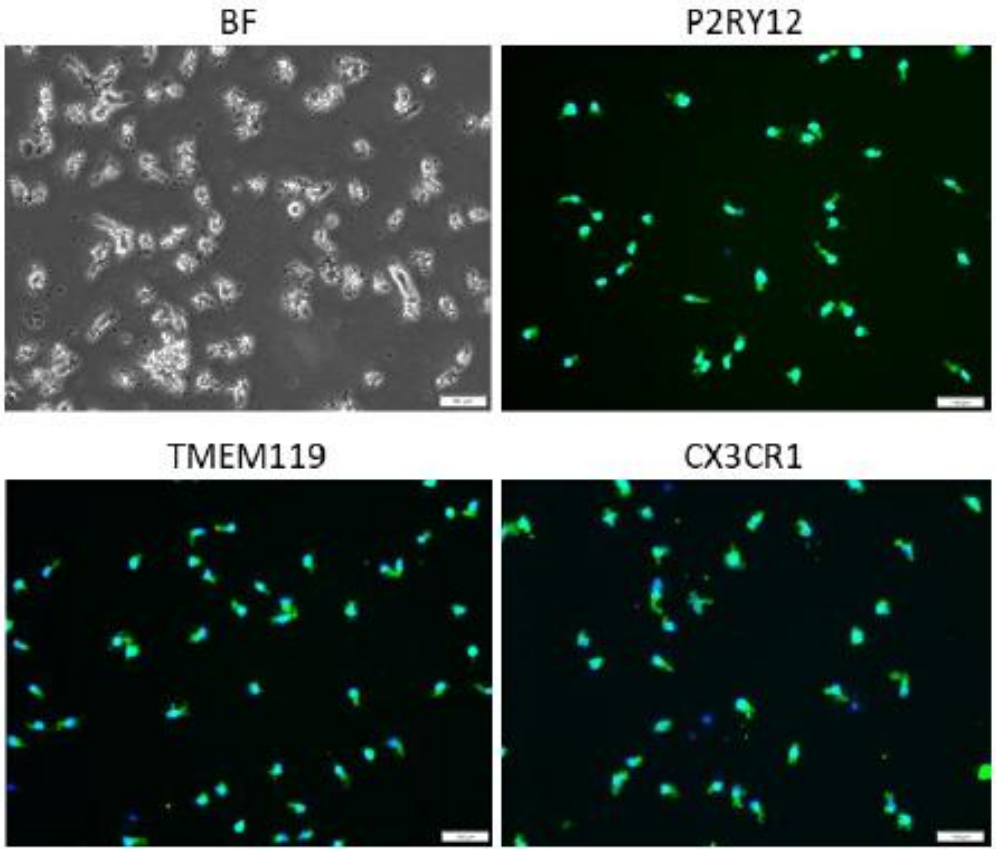

Figure 1. Recovery of cryopreserved ASE-9601A, iPSC-derived microglia (iMGLs). Cryopreserved iMGLs, differentiated from a control iPSC line, were recovered in microglia culture media. The cells were fixed the next day and stained with microglial-specific markers (green), P2RY12, TMEM119, and CX3CR1; counterstain (DAPI; blue) was used to stain the nucleus. BF: Bright field image. Images at 20x magnification.

Related Products

Catalog #	Product	Size
ASE-9600	iPSC-Derived Astrocytes With Media	1 X 10^6 cells/vial
ASE-9598	iPSC-Derived Cortical Neurons With Media	1 X 10^6 cells/vial
ASE-9597	iPSC-Derived Neural Progenitor Cells (NPCs) With Media	1 X 10^6 cells/vial
ASE-9599	iPSC-Derived Dopaminergic Neurons With Media	1 X 10^6 cells/vial
ASE-9602	iPSC-Derived Motor Neurons With Media	1 X 10^6 cells/vial

Technical Support

CONTACT US

For more information or assistance, contact Customer Service at:

- Email: info@appliedstemcell.com
- Direct line: (408) 773-8007

WEBSITE RESOURCES

Visit appliedstemcell.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificates of Analysis
- FAQs
- Product literature
- Complete contact information

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