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Cryopreservation of iPSC-Derived Microglia

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We use this protocol and it's working.

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Abstract

This protocol outlines cryopreservation and thawing procedures for human induced pluripotent stem cell (iPSC)-derived microglia (iMicroglia). The described methods enable the long-term storage of mature iMicroglia in either

 -80 °C freezers or liquid nitrogen tanks, ensuring cell viability upon thawing. By utilizing appropriate cryoprotectants, this protocol facilitates the effective preservation of microglia for future studies, allowing for reproducible experiments and robust recovery after cryopreservation.

Guidelines

o **Time Sensitivity:**

Both freezing and thawing should be done as quickly as possible to minimize cellular damage. After thawing, immediately transfer the contents to  5 mL of prewarmed fresh media.

o **Sterile Environment:**

All work should be conducted in sterile laminar flow hoods (clean bench) designated for human iPSC work. Always use filter tips to minimize the risk of contamination.

Materials

- ☒ DPBS no calcium, no magnesium **Invitrogen - Thermo Fisher Catalog #14190136**
- ☒ Dimethyl sulfoxide (DMSO) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D2650**
- ☒ Accutase **Gibco - Thermo Fisher Scientific Catalog # A1110501**
- ☒ Knockout™ Serum Replacement **Gibco - Thermo Fisher Scientific Catalog #10828028**
- ☒ Bambanker™ Serum Free Cell Freezing Medium **Bulldog Bio Catalog #BB05**
- ☒ Advanced RPMI 1640 Medium **Thermo Fisher Catalog #12633012**
- ☒ GlutaMAX™ Supplement **Thermo Fisher Scientific Catalog #35050061**
- ☒ Recombinant Human IL-34 **Thermo Fisher Scientific Catalog #200-34**
- ☒ Recombinant Human GM-CSF **Thermo Fisher Scientific Catalog #300-03**

o Equipment

- Centrifuge
- P1000, P200, and P10 tips
- 50 mL falcon tubes (Avantor, cat #89039-658)
- Cryogenic storage vials (Corning, cat #431416)
- Cell counting equipment
- Corning CoolCell or Freezing container
-  -80 °C freezer
- Liquid Nitrogen tanks

Troubleshooting

Before start

- Prepare and label the cryogenic vials with the following information: cell line, clone number, day of differentiation, cell number, and freezing date.
- The cell freezing media should be stored at  4 °C before use.



Freezing Procedure

25m

1 Collection of Cells:

25m

- Aspirate the culture media from the T-75 culture flask. Add  5 mL of Accutase to the cells and incubate for  00:20:00 .
- Add 1X phosphate-buffered saline (PBS) to the cells, gently lift them off the surface, and transfer the suspension into a 50 mL centrifuge tube.
- Centrifuge the cells at  1000 rpm, 00:05:00 . Resuspend the cell pellet in 1xPBS and count the cell density with a cell counter.
- Centrifuge the cells at  1000 rpm, 00:05:00 . Carefully aspirate the supernatant and resuspend the cell pellet into either cryoprotective media below at appropriate cell density.
 - 1) KnockOut™ Serum Replacement + 10% Dimethyl sulfoxide (DMSO)
 - 2) Bambanker™ Serum-Free Cell Freezing Medium

2 Storage:

- Aliquot the cell suspension into cryovials (typically  800 μ L per vial) and place them in a Corning CoolCell or similar freezing container, then transfer the container to a  -80 °C freezer.
- For long-term storage, the cryovials can then be transferred to liquid nitrogen tanks after  24:00:00 , once they have stabilized at the  -80 °C temperature. An important feature of Bambanker Cell Freezing Media is its ability to enable the cryopreservation of cells for long-term storage at  -80 °C freezer without stepwise freezing.

Thawing Procedure

2d 0h 5m

3 Preparation:

- Remove the cryovials from liquid nitrogen or the  -80 °C freezer.
- Quickly thaw the cryovials by placing them in a  37 °C water bath, gently swirling to ensure uniform thawing.

4 Culture Recovery:

2d 0h 5m

- After thawing, transfer the cell suspension to a tube containing prewarmed microglia maturation media.



- Centrifuge the cells at  1000 rpm, 00:05:00 to remove the cryoprotectant, which can be toxic to cells.
- Carefully aspirate the supernatant and resuspend the cell pellet in fresh maturation media. Transfer the cells to an appropriate plate or flask to allow them for recovery.
- To recover a cryopreserved culture, the cells need to be cultured for a few hours to  72:00:00 to recover their morphology and functionality.

5 **Post-Thaw Culture:**

- Maintain the cells in a suitable microglia maturation media consisting of Advanced RPMI 1640 Medium, 2 mM GlutaMax, 100 ng/mL IL-34, and 10 ng/mL GM-CSF.
- Change the media every other day and check their viability regularly.

Protocol references

o **Material References**

Navarro et al., LRRK2 G2019S variant is associated with transcriptional changes in Parkinson's disease human myeloid cells under proinflammatory environment. bioRxiv. [Preprint]. 2024 May 31:2024.05.27.594821. doi: 10.1101/2024.05.27.594821).

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