If you have any questions or need assistance, please reach out to technical@bit.bio and we will do our best to support you.

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MATERIALS AND EQUIPMENT

- **ioMicroglia cells (io1021)**
  Cultured to day 10 in a 96-well plate seeded at 37,000 cells/cm² (200 µL/well)
- **comp:MM2x medium**
  As described in the ioMicroglia user manual
- **comp:MM2x+LPS+IFNγ medium**
  As described in Appendix 1
- **96-well U bottom plates**
- **Adhesive film**
- **LPS (50 µg/mL)**
- **IFNγ (50 µg/mL)**
- **Biological safety cabinet**
- **Standard tissue culture wares**
  (pipettes, tips, culture plates)
- **Normoxic cell culture incubator**
  (37°C, 5% CO_2)
- **Vortex**

ioMicroglia

Stimulation for cytokine release

Introduction

Microglia, as residents of the central nervous system, thrive in a complex and heterogeneous environment that is populated with neurons and glial cells. In their capacity as brain-resident immune cells, microglia play a critical role in responding to tissue damage and infection through the release of cytokines and phagocytosis of pathogens.

There is a need for functional and consistent human microglia for neuroimmune research and drug development for neurodegenerative diseases. Current models have constraints such as limitations in supply, complex protocols, or lot variability. All which raise challenges for drug discovery due to the generation of unreliable data and long experimental timelines.

ioMicroglia are human induced pluripotent stem cell (iPSC)-derived microglia, precision reprogrammed using opti-ox™ technology. At 10 days post-revival, they demonstrate maturity reminiscent of primary adult and foetal microglia. Functionally, ioMicroglia secrete proinflammatory and antiinflammatory cytokines (IL-6, TNFα, IL-1b, IL-12p70, IL-8, and IL-10) in response to stimuli such as Lipopolysaccharides (LPS) and Interferon Gamma (IFNγ). The protocol depicted below outlines general guidelines for stimulating cytokine release.

NOTES

- The following protocol recommends general guidelines. We encourage users to optimise the critical steps according to their experimental conditions.

Protocol overview

Step 1.
Make comp:
MM2x + LPS + IFNy

Step 2.
Replace 50% of the medium from day 10 ioMicroglia with comp:
MM2x + LPS+IFNg

Step 3.
Incubate the cells at 37°C for 24 hours

Step 4.
Harvest supernatant for analysis

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Protocol

This protocol is split into two sections:

1. Treatment of cells with LPS and IFNg.

2. Harvesting of cell supernatants for cytokine readout.

1. Treatment of cells with LPS and IFNγ

1.1. Add comp:MM2x+LPS+IFNγ medium and comp:MM2x medium to separate reagent reservoirs.

1.2. Remove 100 µL of expended culture medium from each well.

1.3. Add 100 µL of the comp:MM2x+LPS+IFNγ medium to the treatment wells, for a final volume of 200 µL.

1.4. Add 100 µL of fresh, pre-warmed comp:MM2x medium to the control wells.

1.5. Incubate plates for 24 hours in an incubator at 37°C, 5% CO_2.

2. Harvesting of cell supernatants for cytokine readout

2.1. After the 24 hour incubation, remove plates from the incubator and transfer them to a biological safety cabinet.

2.2. Gently transfer 100 µL of the medium from the culture plates to a fresh 96-well U bottom plate.

2.3. Cover with adhesive film and transfer to the -20°C freezer until ready to analyse on an Meso Scale Discovery (MSD) or ELISA based assay system.

Optional: The attached cells may be fixed for analysis via immunofluorescent staining at a later date.

Note: bit.bio uses an MSD system and examines the following cytokines: IL-6, TNFα, IL-1b, IL-12p70, IL-8, and IL-10.

Appendix 1

Preparation of comp:MM2x+LPS+IFNγ medium

1. Dilute 10 µL LPS stock (50 µg/mL) in 90 µL of comp:MM2x medium.
2. Dilute 10 µL IFNγ stock (50 µg/mL) in 90 µL of comp:MM2x medium.
3. Use the diluted reagents to prepare comp:MM2x+LPS+IFNγ medium as indicated in Table 1 (make 100 µL per test well plus an excess ~10% for volume loss).

Table 1- Preparation of comp:MM2x+LPS+IFNγ medium

Reagent	Stock concentration	Final concentration	comp:MM2x+LPS+IFNγ (for 1000 µL)
comp:MM2x medium	-	-	976 µL
Diluted IFNγ	5 µg/mL	20 ng/mL	4 µL
Diluted LPS	5 µg/mL	100 ng/mL	20 µL