RASTRUM

Protocol

ELISA Assay for RASTRUM™ 3D Cell Models

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Introduction

Assessing the proteins secreted by cells within RASTRUM 3D models can provide data on the response from cells in disease state or drug treatment. Herein, we present a protocol to measure Interleukin-6 (IL-6) in an iPSC-derived astrocyte 3D model exposed to lipopolysaccharide (LPS) using R&D System Human IL-6 Duoset Elisa kit (DY206).[1]

Equipment and reagents required, but not provided

- RASTRUM large plug 3D cell models
- Astrocytes media (astrocyte basal medium (ScienCell), 2 % FBS, astrocyte growth supplement and 10 U/mL penicillin/streptomycin solution)
- Plate sealers: (R&D Systems, Catalog # DY992)
- Human IL-6 Duoset Elisa kit (R&D Systems, DY206)
- Phosphate buffered saline
- Tween 20
- High quality Bovine serum albumin without proteases, binding proteins, soluble receptors
- Microplate reader
- Plate shakers
- Multichannel pipette
- 22°C waterbath

Protocol

1. Print iPSC-derived astrocytes (5 x 10^6 cells/mL) RASTRUM with a density of 15,000 cells/well in a large plug model.
   Note: We recommend adding protease and phosphatase inhibitors to the RIPA buffer to prevent the degradation and dephosphorylation of protein targets.
2. Add 200 µL of astrocytes media to each well and incubate for a minimum of 24 hours at 37°C/ 5% CO_2.
3. Remove media after 24 h of incubation for optional Step 4. Otherwise, continue with Step 5.
4. Drug treatment or stimulus can be done in this step. i.e expose cells to 10 Lipopolysaccharide (LPS) in 200 µL of astrocyte medium per well for 24 h.

   Note: A concentration of LPS at 10 µg/mL is used according to 2D cell culture. 10 µg/mL is sufficient to induce IL-6 release in RASTRUM 3D printed astrocytes.
5. Transfer and combine the cell culture media from two wells of the 96-well plate into a microfuge tube and centrifuge (10,000 g, 1 min).
   Note: Be sure to carefully transfer the cell culture media from wells by ensuring not to disturb the 3D hydrogel models.
6. Carefully transfer the supernatants into fresh microfuge tubes and proceed immediately to ELISA or store at -80°C.

7. Perform IL-6 ELISA according to manufacturer's protocol.

   Note: In this example protocol, IL-6 Duoset ELISA kit is used with slight amendments as follow: Capture antibody is reconstituted with PBS to 2 µg/mL; Detection antibody is diluted to 50 ng/mL; Standard curve is ranged from 0.586-600 pg/mL. Users might need to adjust accordingly depending on target protein concentration.

Protocol

1. Sullivan, M.A., Lane, S.D., Volkerling, A., Engel, M., Werry, E.L. and Kassiou, M., (2022). 3D Bioprinting of Stem Cell-Derived Central Nervous System Cells Enables Astrocyte Growth, Vasculogenesis and Enhances Neural Differentiation/Function. bioRxiv. Published online November 14, 2022.

INVENTIA

Inventia Life Science Operations Pty Ltd
ABN 19 613 078 710
20-22 William Street,
Alexandria, NSW, 2015, AUSTRALIA

info@inventia.life | www.inventia.life