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Value from Innovation

Cellular Dynamics

IL-6 Cytokine Release Assay with iCell® Macrophages 2.0

iCell Lab Note

Introduction.

Studying cytokine release from macrophages is of significant scientific and clinical interest for several reasons, including modeling the human immune response, understanding disease mechanisms, and drug development. IL-6 is a key cytokine involved in various immune and inflammatory responses. Dysregulation of IL-6 production is implicated in numerous conditions, from autoimmune disorders to even cancers. By assessing compound effects on IL-6 release, researchers can identify new drug candidates and optimize dose-response relationships for therapeutic interventions. Implementing human iPSC-derived macrophages can help to overcome many of the challenges associated with using primary monocytes to perform the studies described above. This document provides a technical overview and example data for stimulating iCell Macrophages 2.0 with lipopolysaccharides (LPS) and/or interferon gamma (IFNγ) and then measuring IL-6 cytokine release via various immunoassay technologies.

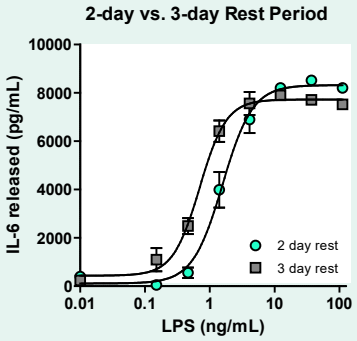


Figure 1. Evaluation of when to run assay. Number of days cells were allowed to rest post-thaw was tested. Overnight LPS stimulation of iCell Macrophages 2.0 starting on either day 2 or day 3 was performed. Robust and dose-dependent release of IL-6 was detected in both cases with EC_{50} values of 1.58 and 0.70 ng/ml, respectively.

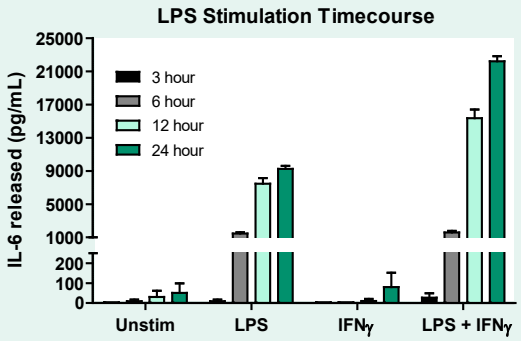


Figure 2. Assessment of LPS stim time. iCell Macrophages 2.0 were stimulated for 3, 6, 12, and 24 hours with either LPS (100 ng/ml), IFNγ (50 ng/ml), or both together. LPS induced robust IL-6 release peaking at 24 hours and co-stimulation with IFNγ augmented cytokine release. IFNγ alone did not trigger any significant response.

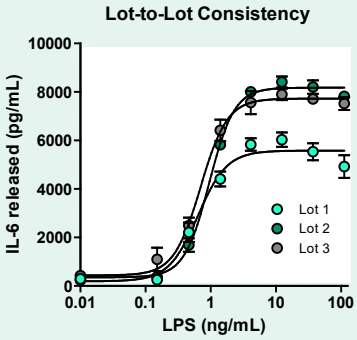


Figure 3. Lot-to-Lot consistency. iCell Macrophages 2.0 from three different batches were compared in the IL-6 cytokine release assay. EC_{50} values from three lots of iCell Macrophages 2.0 were 0.95, 0.55, and 0.70 ng/ml. It is likely that Lot 1 had fewer than 15,000 cells/well to start the assay, resulting in a lower maximum amount of IL-6.

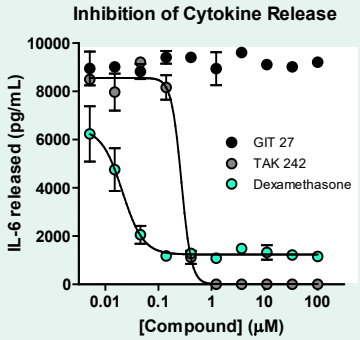
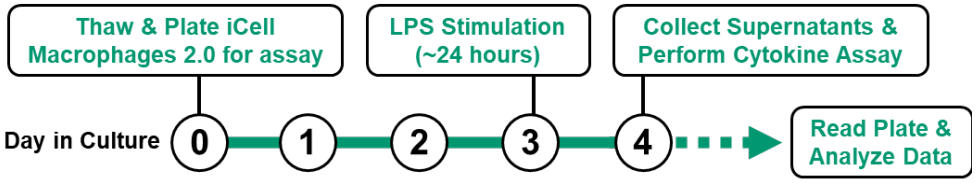
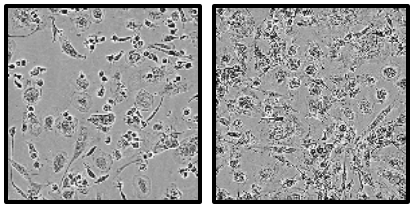


Figure 4. Inhibition of LPS activation. iCell Macrophages 2.0 were pretreated with the compounds above for ~1 hour and then treated with 5 ng/ml LPS for 24 hrs. GIT-27 did not affect IL-6 release, but Dexamethasone and TAK 242 significantly reduced cytokine production, with IC_{50} values of 0.021 and 0.27 µM, respectively.

Assay Workflow Schematic.





untreated +LPS/IFNγ

Methods.

Prepare iCell Macrophages 2.0 media and thaw according to the iCell Macrophages 2.0 User's Guide.

- On Day 0, seed iCell Macrophages 2.0 (200 µl/well) into a 96-well cell culture plate at a density of 30,000 cells/well.
- Incubate iCell Macrophages 2.0 at 37ºC/5% CO_2 for 3 days.
- On Day 3, perform a 50% media change and stimulate with LPS for approximately 24 hours.
- On Day 4, collect cell culture supernatants; immediately test or store at -20 ºC for later analysis.
  - **Note:** The same supernatants may be used for detection of multiple cytokines of interest.
- Follow steps in the Revvity manual to perform the HTRF immunoassay for IL-6 cytokine release.
  - **Note:** Sample amount can be reduced from 16 µl to 8 µl, making the total assay volume required = 10 µl.
- Read fluorescence (665/620 nm) on an HTRF-certified plate reader.
- Correlate HTRF signal intensity to the concentration of IL-6 released (pg/ml) using a standard curve.

Table 1. Materials Needed.

Product	Vendor	Cat. #
iCell Macrophages 2.0 Kit, 01279	FCDI	R1186
LPS from *E. coli* O127:B8 †	Sigma	L4516
Human IFNγ Recombinant Protein †	PeproTech	300-02
HTRF Human IL-6 Detection Kit ◊	Revvity	62HIL06PEG
96-well cell culture microplate †	Greiner	655090
ProxiPlate 384-well, white, low bind †	Revvity	6059480
TAK 242 (TLR4 inhibitor) ‡	Tocris	6587

† Alternate source materials (e.g., reagents and plates) may be substituted but the impact on assay performance is unknown.
◊ Different immunoassay platform technologies (i.e., Lumit® from Promega or ELISA) may also be used to quantify IL-6 release. Please inquire.
‡ Contact Technical Support for details about other compounds tested.

Summary.

This iCell Lab Note provides the basic instructions for performing LPS-induced activation of iCell Macrophages 2.0 and measuring IL-6 release using HTRF assay technology. This cell culture and assay protocol allows for some flexibility when working with iCell Macrophages 2.0, in that other types of cell culture plates, days of rest post-thaw, LPS stimulation times, or even cell densities per well, will still yield robust cytokine release with results similar enough to the representative data presented here. These cells provide a reliable source of human macrophages that can be used for modeling the immune response. IL-6 was used as an example due to its key role in inflammation, but the information in this document is applicable as a guide for testing other cytokines.

Highlights.

iCell Macrophages 2.0 display robust release of IL-6 following stimulation with LPS. Production of this cytokine can be modulated w/ compounds.

The sensitive HTRF assay format detects consistent dose-responses across multiple lots of iCell Macrophages 2.0.

Culture and stimulation protocol can be used with alternate assay platforms or to detect different cytokines.

Scan here to download the iCell Macrophages 2.0 User's Guide.

Contact **Technical Support** (FCDI-Support@fujifilm.com) for more detailed protocol information and supportive data.

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