

InBlood Assay™ – Immunosuppression

Aim

To evaluate in fresh whole blood the capacity of a compound to inhibit cytokine release by activated blood cells.

Biologics or small molecules can be tested in this assay which can be used as a screen for anti-inflammatory or immunosuppressive compounds.

Method

Format: 96-well, reaction volume 100µl

Whole blood: fresh (less than 4h after collection) from healthy adult donors.

Number of donors: 3-6 per plate (see different layout options)

Stimulus: ultrapure LPS 1EU/ml or heat-killed L. monocytogenes (HKLM, 10⁷/ml)

Positive control: Dexamethasone 1µM

Negative controls: unstimulated blood, compound only without stimulus

Incubation: 15min preincubation with compound followed by 4h or 24h stimulation with LPS or HKLM.

Readout: TNF or IL6 measured using HTRF®

Turnaround time

One week from compound receipt (for urgent assays, unformatted results can be communicated within 48h, this service will incur extra charges)

Compound

Must be preferably sent solubilised in saline solution at, at least, 10x the highest concentration to be tested.

Total amount required: at least 1.5x the total amount needed for the assay

The concentrations tested must correspond to a serial dilution of the compound.

Concentrations & replicates: see layout options.

Important note: Blood will be activated by low concentration of endotoxins. Consequently, **the compound must be free of endotoxin to prevent unspecific stimulation of blood.** Endotoxin content can be reduced by filtering the solution containing the compound on 0.2µm PES filters or by using columns like Detoxi-Gel™ (Pierce).

Layout

One compound tested

Nb of donors	Nb of concentrations tested/donor +/- inducer	Replicates
3	6 (+ 2 controls)	2
4	4 (+ 2 controls)	2
6	2 (+ 2 controls)	2

Two compounds tested

Nb de donors	Nb of concentrations tested/compound/donor +/- inducer	Replicates
3	3 (+ 2 controls)	2

Pricing

Base price is for one 96-well plate, one compound tested on blood from 3 donors (highlighted in the table above). Additional fees will be charged for extra donors and extra compounds tested in the same plate.

Results

A research report with estimated IC50 for compounds showing >50% inhibition will be send after completion of the assay.

Limitation

Assays carried out by Blood Assay Solutions are for research purposes only. The results cannot be used directly in dossiers submitted to regulatory authorities to support the application for a diagnosis reagent or a drug to be used in animal and/or human health.

Additional services

The following services are optional and will incur extra charges.

- Long term storage of supernatants at -80°C for future use.
- Additional readout by HTRF or ELISA on stored supernatants

Suggested plate layout for 3 donors, samples in duplicate.

