

## 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 0.1EU/ml or heat-killed L. monocytogenes (HKLM, 10<sup>7</sup>/ml)

Positive control: Dexamethasone 10µM

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 of 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.

