

InBlood Assay™ – Immunoactivation

Aim

To evaluate in fresh whole blood the capacity of a compound to activate blood cells.

A cytokine release in this assay reflects the immunogenicity of the compound. This can be due to the presence of contaminants, an adjuvant-like effect, pro-inflammatory properties of the compound, a risk of cytokine release syndrome if the tested compound is a biologic.

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)

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

Negative control: unstimulated blood

Incubation: 4h or 24h

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(s).

Concentrations & replicates: see layout options.

Important note: Blood will be activated by low concentration of endotoxins. Consequently, **unless the aim of the study is to evaluate the presence of endotoxins in the compound, 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	Replicates
3	8 (+ 2 controls)	3
4	10 (+ 2 controls)	2
4	6 (+ 2 controls)	3
6	6 (+ 2 controls)	2
6	3 (+ 2 controls)	3

Two compounds tested

Nb of donors	Nb of concentrations tested/compound/donor	Replicates
3	6 (+ 2 controls)	2
4	4 (+ 2 controls)	2
6	2 (+ 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 EC50 for compounds reaching saturation will be send after completion of the assay.

Limitations

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 triplicate.

