ELISPOT

PREPARE PLATES AND COAT WITH PRIMARY ANTIBODY

Rinse 96 well ELISPOT plates in 70% ethanol for 30 seconds.
25 µl per well.

Coat ELISPOT plate with capture antibody diluted in PBS.
Incubate at 4°C overnight.

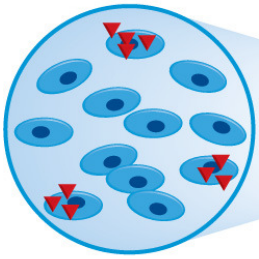
Wash wells 3X in PBS.

Block plates by adding 100 µl 2% milk per well. Incubate 2 hr.
Wash wells 1X in PBS.

INCUBATE WITH CELLS

Add 1 x10^5 to 2 x10^5 cells per well.
Optimize according to cell type and the percentage of cells expected to secrete the protein.
Cells should be >95% viable.

Stimulants such as LPS mitogen may be required for analysis of protein (e.g. cytokine) expression.
During incubation, the cells will secrete the protein/cytokine.



Culture overnight at 37°C in CO_2 incubator.
Cells must remain stationary during incubation.
The culture conditions such as cell number and incubation time may require optimization.

WASH CELLS AWAY

Incubate 10 min with PBS 0.1% Tween 20.
Wash plates 3X with PBS 0.1% Tween 20.

DETECTION ANTIBODY: INCUBATION FOR DETECTION OF SECRETED PROTEIN.

Add 100 µl per well of conjugated detection antibody (diluted in PBS 1% BSA).
Incubate 1-2 hr at RT.
Optimization of antibody concentration and incubation time may be required.

ENZYMATIC DETECTION
Follow manufacturer recommendations

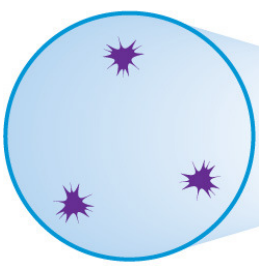
Add 100 µl of streptavidin-alkaline phosphatase (diluted 1:5000).
Seal the plate and incubate 1 hr at 37°C.

Wash 3X with PBS 0.1% Tween 20.
Remove residual buffer by tapping gently on absorbent paper.

Add 100 µl of ready to use BCIP-NBT buffer in wells.
Incubate for 2-10 min at RT. Monitor spot formation visually.

Colored product

Substrate eg BCIP/NBT



Wash plates in distilled running water to stop the spot formation (take the base of the plate and wash both sides of the membrane).

Dry the plates and let membranes dry completely.

Read on ELISPOT plate reader with applicable software to analyse results.

READ AND ANALYSE

Key

- Capture antibody
- Cells
- Secreted protein/cytokine
- Detection antibody
- Biotin
- Streptavidin
- Colored product

BCIP: 5-bromo-4 –chloro-3´ indolyphosphate p – toluidine salt

NBT: Nitro blue tetrazolium chloride

Colored product: 5-5´-dibromo 4,4´ dichloro indigo-white