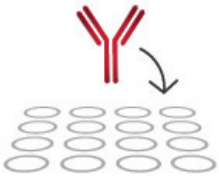
ELISpot Step-by-Step



1. Antibody coating

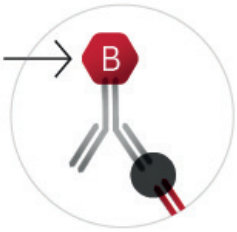
Cytokine-specific monoclonal capture antibodies are immobilized on an ethanol-treated PVDF membrane plate.

2. Cell incubation

Cells are added to the wells in the presence or absence of activating stimuli and then incubated to allow for cytokine secretion.

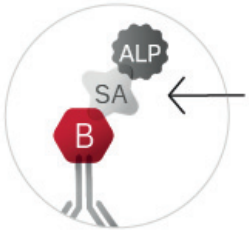
3. Cytokine capture

Secreted cytokines bind to the capture antibodies on the membrane immediately surrounding the activated cells.



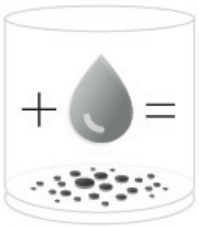
4. Detection antibodies

Following removal of cells and washing of the wells, biotinylated cytokine-specific detection antibodies are added to the wells.



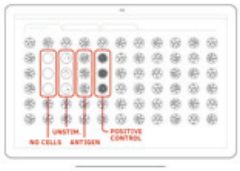
5. Streptavidin-enzyme conjugate

To enable the formation of spots on the membrane, a streptavidin-enzyme conjugate is added to the wells.



6. Addition of substrate

Colorimetric substrate is added to the wells and will form an insoluble precipitate when catalyzed by the enzyme; a visible representation of cytokine release by a single activated cell.



7. Analysis

Spots are counted in an automated ELISpot reader or under a dissection microscope, and the frequency of secreting cells is calculated.