

Human IL-1 β ELISpot

With precoated plates



856.101.001 PC 1x96 wells
856.101.005 PC 5x96 wells

Intended use

The ELISpot assay is designed to enumerate cytokine producing cells in a single cell suspension. This method has the advantage of requiring a minimum of *in-vitro* manipulations allowing cytokine production analysis as close as possible to *in-vivo* conditions in a highly specific way. This technique is designed to determine the frequency of cytokine producing cells under a given stimulation, and the follow-up of such frequency during a treatment and/or a pathological state. ELISpot assay constitutes an ideal tool in the TH1 / TH2 response, vaccine development, viral infection monitoring and treatment, cancerology, infectious diseases, autoimmune diseases and transplantation.

Diaclone ELISpot assay is based on sandwich immuno-enzyme technology. Cell secreted cytokines or soluble molecules are captured by coated antibodies avoiding diffusion in supernatant, protease degradation or binding on soluble membrane receptors. After cell removal, the captured cytokines are revealed by tracer antibodies and appropriate conjugates.

Reagent provided

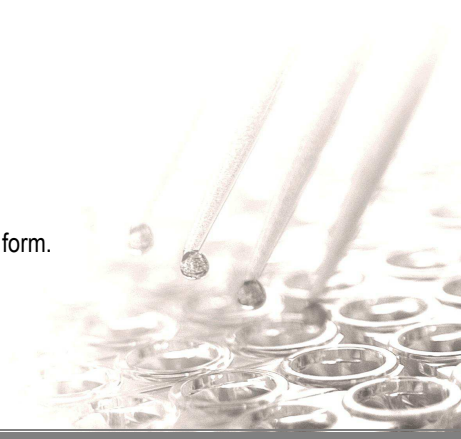
- Precoated 96 PVDF-bottomed-well plates (5)
- Biotinylated detection antibody (lyophilised, resuspend in 0.55mL)
- Streptavidin - Alkaline Phosphatase conjugated (50 μ L)
- Bovine Serum albumin (1 g)
- Ready-to-use substrate buffer (50mL)

Please note for 1 x 96 demo kits Biotinylated detection antibody is provided in liquid form.

Store all reagents at 4°C.

Materials / Reagents not provided

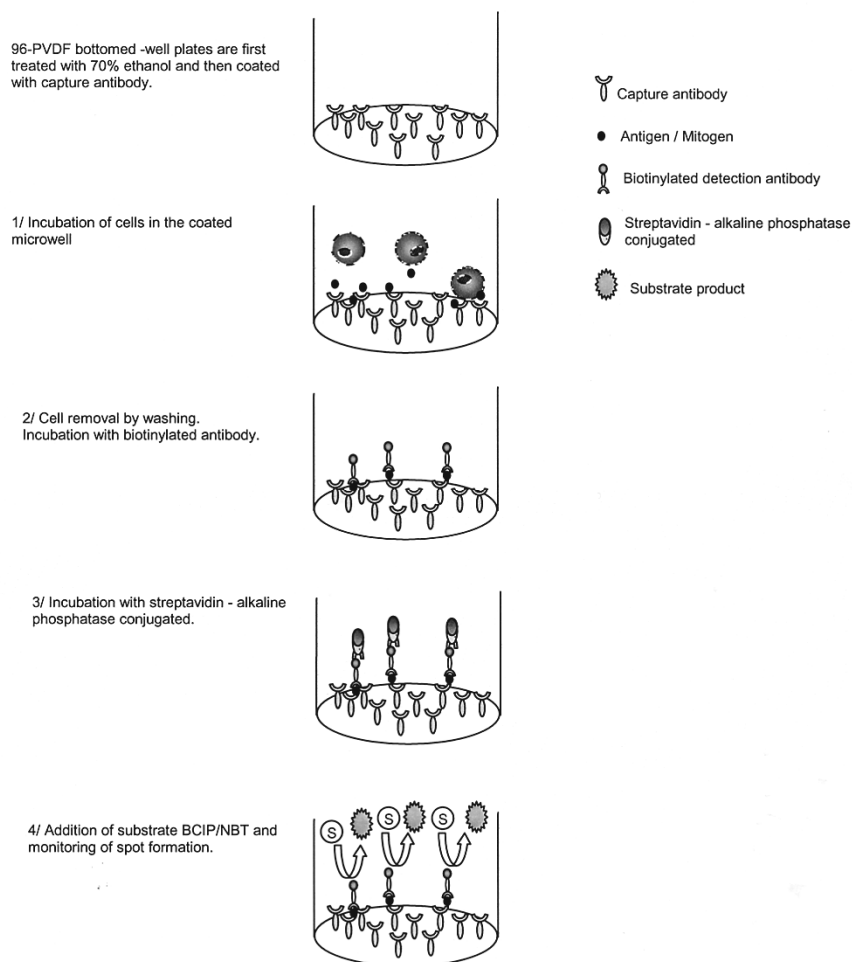
- Cell culture media
- CO₂ incubator
- Tween 20
- Phosphate buffered saline



Principle of the method

After cell stimulation, locally produced cytokines are captured by a specific monoclonal antibody. After cell lysis, trapped cytokine molecules are revealed by a secondary biotinylated detection antibody, which is in turn recognised by streptavidin conjugated to alkaline phosphatase. PVDF-bottomed-well plates are then incubated with BCIP/NBT substrate. Colored "purple" spots indicate cytokine production by individual cells.

Procedure Summary



Assay control

IL-1 β production by PBMC upon stimulation by LPS. This protocol is given as a suggestion

Dilute PBMC in culture media (e.g. RPMI 1640 supplemented with 2mM L-glutamine and 10% heat inactivated fetal calf serum) containing 1 μ g/ml LPS (Sigma, Saint Louis, MO). Distribute 1.10^5 to 1.10^4 cells in antibody coated PVDF-bottomed-wells and incubate for 10-15 hours in an incubator. Note that as IL-1 β is mainly produced by monocytes/macrophages amongst PBMC. Spontaneous release of IL-1 β occurs while the cells adhere to the PVDF membrane.

For other stimulators incubation times may vary, depending on the frequency of cytokine producing cells, and should be optimised in each situation.

Reagent Preparation

▪ **Detection antibody**

Reconstitute the lyophilised antibody with 0.55mL of distilled water. Gently mix the solution and wait until all the lyophilised material is back into solution.

If not used within a short period of time, reconstituted detection antibody should be aliquoted and stored at -20°C. In these conditions the reagent is stable for at least one year.

▪ **Streptavidin alkaline phosphatase**

For optimal performance prepare the Streptavidin-AP dilution immediately prior to use.

For 1 plate dilute 10µl of Streptavidin-AP conjugate into 10 mL Dilution Buffer and mix well.

Do not keep this solution for further experiments.

▪ **Phosphate buffered saline (10X Concentrate solution).**

For 1 liter weight : 80g NaCl ; 2g KH₂PO₄ ; 14.4g Na₂HPO₄ 2H₂O. Add distilled water to 1 liter. Check that pH is comprised between 7.4 +/- 0.1. **Dilute the solution to 1X before use.**

▪ **1% BSA in PBS**

For one plate dissolve 0.2 g of BSA in 20 mL of 1X diluted PBS.

▪ **0.1% Tween in PBS**

For one plate dissolve 100µl of Tween 20 in 100 ml of 1X diluted PBS.

Elispot Procedure

1. Incubate precoated PVDF-bottomed-well plate with 100µl / well of PBS for 10mn at room temperature.
2. Empty wells by flicking the plate over a sink and tapping it on absorbent paper.
3. Dispense into wells 100 µl of cell suspension containing the appropriate number of cells and appropriate concentration of stimulator. Cells may have been previously in-vitro stimulated (Indirect ELISPOT). Cover the plate with a standard 96-well plate plastic lid and incubate cells at 37°C in a CO₂ incubator for an appropriate length of time (10-15 hours).
During this period do not agitate or move the plate.
4. Empty wells by flicking the plate over a sink and gently tapping it on absorbent paper.
5. Distribute 100µl of PBS-0.1% tween 20 in wells and let sit for 10 min at +4°C.
6. Wash wells three times with PBS-0.1% tween 20.
7. For 1 plate dilute 100µl of reconstituted detection antibody into 10 mL of PBS containing 1% BSA. Distribute 100µl in wells, cover the plate and incubate 1 hour 30 min at room temperature.
8. Empty wells and wash three times with PBS-0.1% tween 20.
9. Distribute 100 µl of streptavidin-Alkaline phosphatase dilution (1/1000) in each well. Seal the plate and incubate for 1 hour at room temperature.
10. Empty wells and wash three times with PBS-0.1% tween 20.
11. Peel off the plate bottom and wash three times both sides of the membrane under running distilled water. Remove all residual buffer by repeated tapping on absorbent paper.
12. Distribute 100µl of ready-to-use BCIP/NBT buffer in wells.
13. Let the reaction go for about 2-10 min at room temperature. Monitor spot formation visually.
14. Rinse three times both side of the membrane under running distilled water.
15. Dry wells. Read spots. Note that spots may become sharper after one night at +4°C.
Store the plate at room temperature away from direct light.

Notes and recommendations

Cell stimulation

1. Cells can either be stimulated directly in the antibody coated wells (Direct) or, first stimulated in 24 well plates or flask, harvested, and then plated into the coated wells (Indirect).
The method used is dependent on 1) the type of cell assayed 2) the expected cell frequency. When a low number of cytokine producing cells are expected it is also advised to test them with the direct method, however, when this number is particularly high it is better to use the indirect ELISpot method. All the procedure beyond the stimulation step is the same whatever the method (direct/indirect) chosen.

Substrate

2. BCIP/NBT buffer is potentially carcinogenic and should be disposed off appropriately. Caution should be taken while handling this reagent. Always wear gloves.