Human IL-1β ELISpot Kit
PVDF Format

	Without Plates	With Plates	With Sterile Plates	Quantity
Catalog Nos.	856.101.001	856.101.001P	856.101.001S	1 x 96 tests
	856.101.005	856.101.005P	856.101.005S	5 x 96 tests
	856.101.010	856.101.010P	856.101.010S	10 x 96 tests
	856.101.015	856.101.015P	856.101.015S	15 x 96 tests
	856.101.020	856.101.020P	856.101.020S	20 x 96 tests

Intended use

ELISpot is a highly specific immunoassay for the analysis of cytokine and other soluble molecule production and secretion from T-cells at a single cell level in conditions closely comparable to the *in-vivo* environment with minimal cell manipulation. This technique is designed to determine the frequency of cytokine producing cells under a given stimulation and the comparison of such frequency against a specific treatment or pathological state. The ELISpot assay constitutes an ideal tool in the investigation of Th1 / Th2 responses, vaccine development, viral infection monitoring and treatment, cancerology, infectious disease, autoimmune diseases and transplantation.

Utilizing sandwich immuno-enzyme technology, ELISpot assays can detect both secreted cytokines and single cells that simultaneously produce multiple cytokines. 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.

This kit has been configured for research use only and is not to be used in diagnostic procedures.

Reagents provided (Contents shown for 5 x 96 test format)

- 96 well PDVF bottomed plates (5 if ordered)
- Capture Antibody (0.5 ml supplied sterile)
- Biotinylated detection antibody (lyophilized, resuspend in 0.55 ml)
- Streptavidin-Alkaline Phosphatase conjugate (50 µl)
- Bovine Serum Albumin (BSA)
- Ready to use BCIP/NBT substrate buffer (50 ml)

Please note for 1x96 demo kits, Biotinylated detection antibody is provided in liquid form.
Store all reagents at 4°C except plates which should be stored at room temperature.

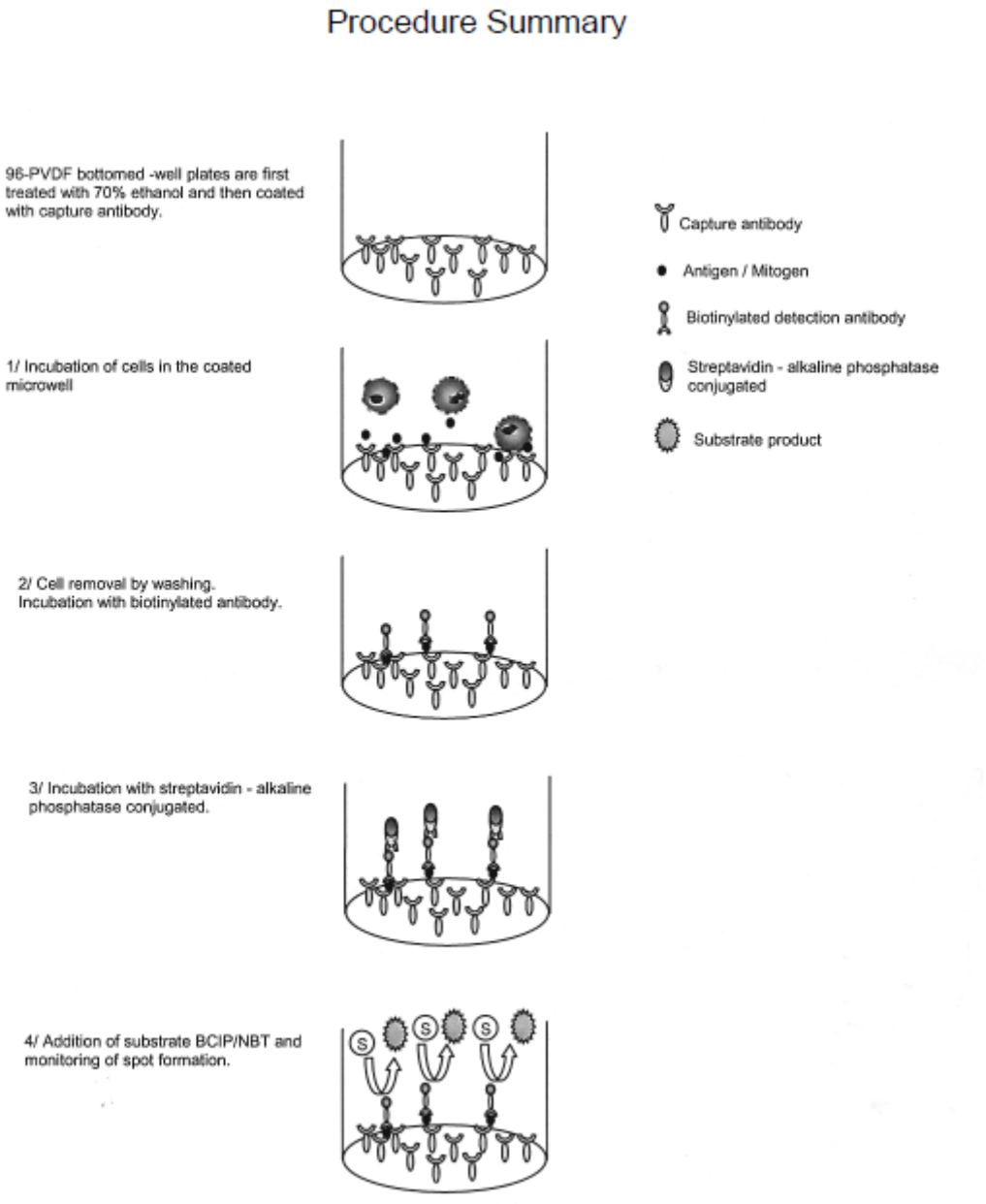
Materials/Reagents required but not provided

- 96 PVDF-bottomed-well plates.
- Cell culture media
- CO_2 incubator
- 70% ethanol
- 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 recognized 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.



Assay control

IL-1β 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 3g/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-1β is mainly produced by monocytes/macrophages amongst PBMC. Spontaneous release of IL-1b 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 optimized in each situation.

Reagent Preparation

- **Detection antibody**

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

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

Please note for the 1 X 96 wells, Biotinylated detection antibody is provided in liquid form.

- **Streptavidin alkaline phosphatase**

Dilute 1/1000 in PBS 1% BSA.

DO NOT KEEP THE DILUTIONS FOR FURTHER EXPERIMENTS

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

For 1 liter weight: 80g NaCl ; 2g KH_2PO_4 ; 14.4g Na_2HPO_4 $2H_2O$. 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.

- **0.05% Tween in PBS**

For one plate dissolve 50 µl of Tween 20 in 100 ml of 1X diluted PBS.

- **35% ethanol in water**

For one plate mix 3.5 ml of ethanol with 6.5 ml of distilled water.

Elispot Procedure

1. Incubate PVDF-bottomed-well plates with 25 µl / well of 35% ethanol for 30 sec at room temperature.
2. Empty wells and wash three times with 100 µl / well of PBS.
3. Pipette 1003l of capture antibody in 10 mL of PBS. Mix and dispense 100 µl into each well, cover the plate and incubate overnight at +4°C.
4. Empty wells and wash once with 100 µl of PBS.
5. Dispense 100 µl of RPMI 10% FCS into wells, cover and incubate for 2 hours at room temperature.
6. Empty wells by flicking the plate over a sink and tapping it on absorbent paper.
7. Wash plate once with PBS.
8. 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 CO_2 incubator for an appropriate length of time (10-15 hours).
   During this period do not agitate or move the plate.
9. Empty wells by flicking the plate over a sink and gently tapping it on absorbent paper.
10. Distribute 100 µl of PBS-0.0.5% tween 20 in wells and let sit for 10 min at +4°C.
11. Wash wells three times with PBS-0.05% tween 20.
12. For one plate dilute 100 µl of reconstituted detection antibody into 10 mL of PBS containing 1% BSA. Distribute 100 µl in wells, cover the plate and incubate 1 hour 30 min at room temperature.
13. Empty wells and wash three times with PBS-0.05% tween 20.
14. For one plate dilute 10 µl of streptavidin-Alkaline phosphatase conjugate into 10 mL of PBS-1% BSA. Distribute 100 µl of the dilution in wells. Seal the plate and incubate for 1 hour at room temperature.
15. Empty wells and wash three times with PBS-0.05% tween 20.
16. 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.
17. Distribute 100 µl of ready-to-use BCIP/NBT buffer in wells.
18. Let the reaction go for about 5-20 min at room temperature. Monitor spot formation visually.
19. Rinse three times both side of the membrane under running distilled water.
20. 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.