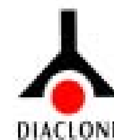
Human IL-13 Elispot

Matched Antibody Pair



869.120.010 10x96 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.

Pair kit contents for 10 plates

- **Capture antibody**, clone B-B13: 2 vials of 0.50 ml.
  The antibody is supplied sterile and does not contain any preservative.
  We strongly advice to pipette it sterily.

- **Biotinylated detection antibody**, clone B-P6: 2 vials lyophilised.

Store the antibodies at 4°C

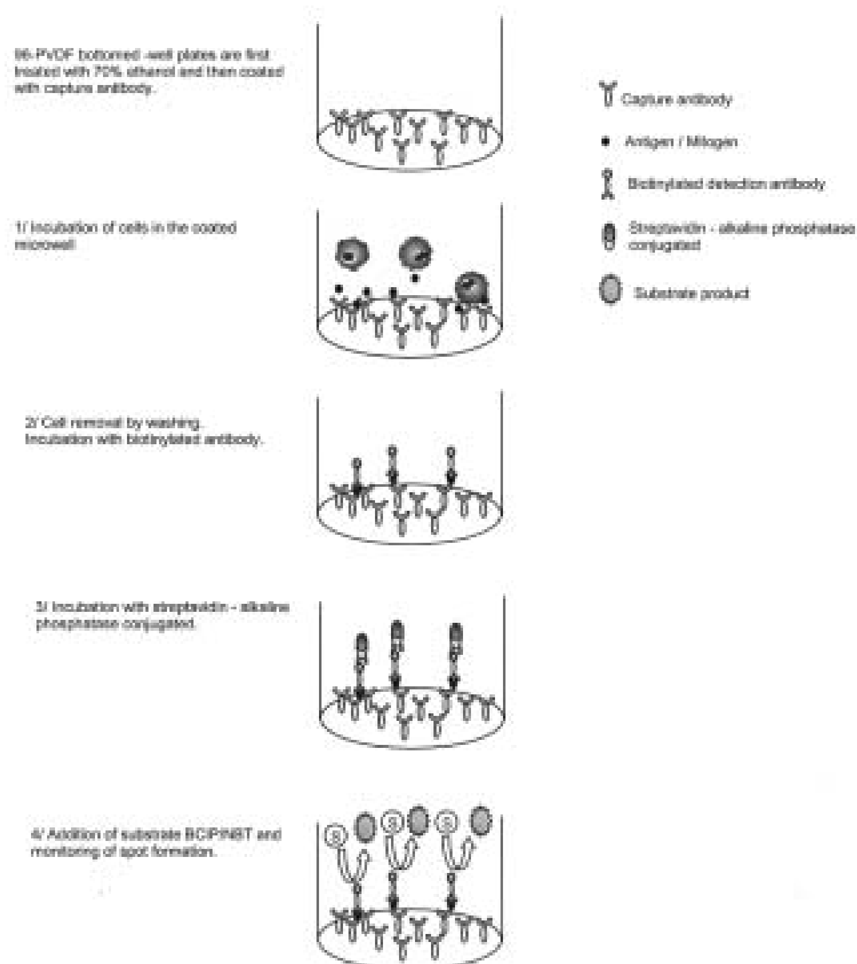
Materials / Reagents not provided

- 96 PVDF-bottomed-well plates. We recommend Millipore MultiScreen plates cat # MAIPN/S4510 or cat # MSIPN/S4510
- Cell culture media
- CO_2 incubator
- 70% ethanol
- Tween 20
- Phosphate buffered saline
- Streptavidin - Alkaline Phosphatase conjugated
- Bovine Serum albumin
- Dry skimmed milk: non sterile Elispot / Liquid sterile milk: sterile Elispot
- Substrate Solution (BCIP/NBT)

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-13 production by Th2 clones upon stimulation by PMA and ionomycin. This protocol is given as a suggestion

Dilute T cells in culture media (e.g. RPMI 1640 supplemented with 2mM L-glutamine and 10% heat inactivated fetal calf serum) containing 1 ng/ml PMA and 500 ng/ml ionomycin (Sigma, Saint Louis, MO). Distribute 2.10^4 to 5.10^4 cells in antibody coated PVDF-bottomed-wells and incubate for 10-15 hours in an incubator.

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**

Dilute 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.**

- **2% dry skimmed milk in PBS**

For one plate dissolve 0.2 g of powder in 10 ml of 1X diluted PBS.

- **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.

- **70% ethanol in water**

For one plate mix 7 ml of ethanol with 3 ml of distilled water.

Recommended Elispot Procedure

1. Incubate PVDF-bottomed-well plates with 25µl / well of 70% ethanol for 30 sec at room temperature.
2. Empty wells and wash three times with 100 µl / well of PBS.
3. Pipette 100 µl 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 2% dry skimmed dry in PBS 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-0.1% tween 20 in wells and let sit for 10 min at +4°C.
11. Wash wells three times with PBS-0.1% tween 20.
12. For 1 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 37°C.
13. Empty wells and wash three times with PBS-0.1% tween 20.
14. Distribute 100 µl of diluted streptavidin-Alkaline phosphatase in each well. Seal the plate and incubate for 1 hour at 37°C.
15. Empty wells and wash three times with PBS-0.1% tween 20. Remove all residual buffer by repeated tapping on absorbent paper.
16. Distribute 100 µl of ready-to-use BCIP/NBT buffer in wells.
17. Let the reaction go for about 2-10 min at room temperature. Monitor spot formation visually.
18. Rince wells three times with distilled water.
19. 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.

Plates

3. While using MAIPN4510 or MAIPS4510 plates, during the incubation steps, reagents can leak through the membrane by capillary action. As this liquid is not properly removed by washing it might increase the background signal. To avoid this we recommend following the procedure detailed below:
   - Peel off the plate bottom at the end of step 15
   - Wash both sides of the membrane under running distilled water.
   - Remove all residual buffer by repeated tapping on absorbent paper.

   For the following incubation in step 16, as the plate bottom has been removed, we suggest that the plate be placed on an empty 96-well plate used as a support. After substrate distribution and incubation (step 16& 17), we recommend to complete the step 18 with a complementary washing of the back of the membrane under running distilled water.