

ELISPOT Procedure

Recommend use with 16 well ELISPOT Slides

Cells can 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 secretion frequency and 3) number of cytokine producing cells expected. Following stimulation the detection method is the same.

1. Pre-incubate 16 well ELISPOT Slides with 100 μ l of 70% ethanol for 10 min at room temperature.
2. Empty wells by flicking the 16 well ELISPOT Slide over a sink and tapping it on absorbent paper and wash three times with 100 μ l of PBS.
3. Dilute capture antibody according to kit instructions and dispense 100 μ l into each well. Incubate 4 hours to overnight at +4°C.
4. Empty wells and wash once with 100 μ l of PBS.
5. Dispense 100 μ l of 2% skimmed dry milk or other blocker in PBS into wells. Cover and incubate for 1 hour at room temperature.
6. Wash 16 well ELISPOT Slide once with PBS.
7. Dispense into wells, 100 μ l 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 and incubate cells at 37°C in a CO₂ incubator for an appropriate length of time (15-20 hours). During this period do not agitate or move the 16 well ELISPOT Slide.
8. Empty wells by flicking 16 well ELISPOT Slide over a sink and gently tapping it on absorbent paper.
9. Dispense 100 μ l of PBS-0.1% Tween 20 in wells and let sit for 10 min at +4°C.
10. Wash wells three times with PBS-0.1% Tween 20.
11. Dilute 100 μ l of reconstituted detection antibody into PBS containing 1% BSA. Dispense 100 μ l in wells, cover and incubate 30-60 min at 37°C.
12. Empty wells and wash three times with PBS-0.1% Tween 20.
13. Detection can be performed using streptavidin-Alkaline phosphatase conjugate in PBS-1% BSA, followed by washing and addition of substrate.
14. Rinse wells three times with distilled water.
15. Dry wells. Read spots.
16. Upper structures may be removed from both plastic and glass slides for storage. The sealing gasket may be removed cleanly from the plastic slide but not the glass slides. After removal of upper structures, slides can be stored at room temperature away from direct light.

EliSpot Slides are for Laboratory Use Only