ELISPOT: Frequently Asked Questions

What are the differences between BD ELISPOT products?

We offer matched ELISPOT antibody pairs, ELISPOT sets, and ELISPOT kits. See the following table for the components for each product type.
http://www.bdbiosciences.com/reagents/elispot/index.jsp

Components	Antibody Pair	ELISPOT Set	ELISPOT Kit
BD ELISPOT 96-well plates	Millipore MultiScreen plates S2EM004M99	10 uncoated	2 antibody-coated and blocked
Capture antibody	For 5 plates	For 10 plates	Pre-coated on plates
Detector antibody (biotin-conjugated)	For 5 plates	For 10 plates	For 2 plates
Streptavidin-HRP	Cat. No. 557630	For 10 plates	For 2 plates
AEC chromogen	Cat. No. 551951	Cat. No. 551951	For 2 plates
Buffers	See TDS	See TDS	For 2 plates
Usage information	Certificate of analysis and protocol	Certificate of analysis and manual	
Key features	• User setup assay with optimized antibody pair • Flexibility for modifying the protocol	• User setup assay with optimized antibody pair • Validated combination of plates and reagents	• Ready to run • Maximum convenience • Premium performance of an optimized reagent system

What type of membrane is used by the ELISPOT plates?

We used Immobilon-P hydrophobic PVDF plates from Millipore (S2EM004M99). The fractal surface and hydrophobic properties of PVDF membranes are ideal for the binding of most coating antibodies and substrates.

Should I pre-wet ELISPOT membrane plates?

Although we do not suggest pre-wetting plates in our protocol, pre-wetting might help ensure coating efficiency in some instances. Pre-wet plates with 70% ethanol, wash well with PBS three times afterwards, and then follow the recommended protocol.

How long can I store coated wet plates?

We recommend coating plates at 4°C overnight, blocking plates at room temperature for 2 hours, and using the plates for ELISPOT assays immediately according to protocol. We do not recommend leaving blocking buffer on plates for storage.

How many cells should I seed?

The optimal cell number for each cell type and preparation need to be determined for each assay. Upon stimulation, the optimal cell concentration per well should be determined separately. We recommend

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determining the optimal cell and stimulant concentration by making serial two- to four-fold dilutions. For example, we seeded 2.5 x 10^3 human PBMCs/well (for human IFN-γ secreting cells) for our QC testing specifications for the BD ELISPOT Human IFN-γ ELISPOT kit (Cat. No. 552138). We seeded 3 x 10^3 human PBMCs/well (for human granzyme B secreting cells) for our QC testing specification for the BD ELISPOT Human Granzyme B ELISPOT kit (Cat. No. 552573).

Do I need to wash cells before seeding?

Yes. Wash cells thoroughly prior to cell seeding to avoid the carryover of natural cytokines made by the cells in a primary culture, or of recombinant proteins that have been added exogenously.

How long should cells be incubated for optimal secretion?

Optimal incubation periods are dependent on the type of cells, stimulant, and protein of investigation. Each cytokine has its own specific kinetics and optimal incubation periods may vary. For example, for human IFN-γ secreting cells (information from lot specific TDS for 552138), we stimulated human PBMCs with 5 ng/mL PMA and 500 ng/mL ionomycin for 24 hours. For human granzyme B secreting cells (information from lot specific TDS for 552573), we stimulated human PBMCs with immobilized anti-human CD3 antibody (Cat. No. 555329) 10 µg/mL and 2 µg/mL soluble anti-human CD28 (Cat. No. 555725), 10 ng/mL recombinant human IL-2 (Cat. No. 554603), and 50 ng/mL recombinant human IL-4 (Cat. No. 554605) for 48 hours. The cells were washed, then cultured in medium containing 10 ng/mL recombinant human IL-2 and 50 ng/mL recombinant human IL-4 for another 48 hours. Finally, the cells were harvested, washed, and re-stimulated with 5 ng/mL PMA and 500 ng/mL ionomycin for 15 hours.

Why do I get irregular or fuzzy spots?

T cells can actively migrate during incubation. Activated T cells can migrate rapidly, leaving a cytokine “trail.” If the plates are shaken or moved during the capture period, the cells will roll, leaving streaks and irregularly shaped spots. To avoid streaks and fuzzy spots, do not move or disturb the incubator during the cell culture period.

How do I avoid uneven spot distribution within a well?

Mix cells gently to ensure homogenous cell distribution before dispensing cells into wells.

Can I stack plates in the incubator?

To ensure even temperature, we do not recommend stacking ELISPOT plates during incubation.

What is the best way to wash the plates?

Wash both sides of the membrane with distilled water before and after color development. Some reagents might leak through the membrane into the base of the plate, which can cause high background if not washed away.

What is the best way to dry the plates after development?

Place the plates in a flow hood overnight. Do not dry the plates at temperature higher than 37°C because the membrane will crack. Longer drying times may help increase the contrast between background and spots.

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