



PeliSPOT Pair

370 tests (> 3 plates)

PRODUCT INFORMATION

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I. INTENDED USE

This antibody pair is suitable to perform an ELISPOT assay for the quantification of the frequency of human cells secreting the protein of interest.

II. CONTENTS

This product contains sufficient material for 370 tests.

Component	Volume	Cap colour	Handling
Coating antibody	190 μ l	Red	Dilute 1:100 in PBS
Biotinylated antibody	375 μ l	Yellow	Dilute 1:100 in dilution buffer

III. PRECAUTIONS FOR USE

1. PeliSPOT Pair is intended for research purposes only, ingredients are not for in vivo use.
2. Handle all blood, tissue and cell samples with care to prevent transmission of infections.
3. Sodium azide inactivates HRP, do not use sodium azide containing solutions in combination with this enzyme.
4. Do not use PBS tablets for coating.
5. Centrifuge all vials before use (1 minute at 3,000 x g).
6. The supplied reagents have been tested and approved in combination with the general assay protocol (see section VI)
7. **Aseptic Procedures; Use sterile buffers and aseptic conditions; use laminar flow hood for procedures.**
8. The supplied reagents have only been tested and approved for use on MSIPS45 microplates from Millipore.
Make sure not to scrape the bottom of the wells and do not let the membrane dry out.
* in combination with a single cell culture tray, Millipore MAMC S01

IV. CELL PREPARATIONS AND STIMULI

Freshly isolated or frozen peripheral blood mononuclear cells (PBMC) as well as cell lines or cultured cells can be used. After being drawn, anti-coagulated blood is kept at room temperature (18 to 25 °C) for a maximum of 24 hours. PBMCs are isolated from venous blood by density centrifugation according to the manufacturer's protocol. Wash the cells twice with cell culture medium.

Add a mitogen as a positive secretion control, for example PMA (1 ng/ml) and Ionomycin (0.5 µg/ml). Wells that exceed the number of 2.5×10^4 mitogen-stimulated cells per well are generally not countable due to high protein secretion.

The optimal concentration of the antigen during antigen-specific stimulation with e.g. peptides and the optimal number of cells per well depend on the cell type and excretion marker and, therefore, they have to be determined by the investigator. Start with a maximal cell number of 2×10^5 cells per well and an antigen concentration between 1 and 10 µg/ml.

V. RECOMMENDED REAGENTS AND BUFFERS*

PBS*	: 10 mM Phosphate buffered saline, pH 7.2-7.4
Cell culture medium*	: IMDM or RPMI-1640 with 5% FCS, 2 mM Glutamine 100 U/ml Penicillin, 100 µg/ml Streptomycin
Wash buffer*	: PBS + 0.005% Tween-20; 0.2 µm filtered
Microtiterplate*	: Preferably Millipore MSIPS45

* For aseptic conditions these components should always be used sterile

Available at Sanquin Reagents:		order number
Dilution buffer	: PeliSPOT buffer	M2540
Enzyme conjugate	: Streptavidin poly-HRP	M2051
Substrate	: TMB for PeliSPOT	M2521

VI. GENERAL ASSAY PROTOCOL

This is a suggestion only, optimal conditions should be determined experimentally.

Check the included specification sheet for cytokine dependent modifications

Day 1 Aseptic Procedures

1. Activate the PVDF membrane of the microplate with 70% ethanol for 60 seconds 15 µl for MSIP plates and for MAIP plates: 100 µl 96% ethanol for 10 minutes at room temperature.
2. Rinse once with distilled water and wash once with PBS.
3. Dilute the coating antibody 1:100 in PBS and add 50 µl per well.

4. Incubate overnight at 2 to 8°C.

Day 2 Aseptic Procedures

5. Wash the microplate five times with wash buffer.
6. Add the cell suspension and cell stimulus of interest.
7. Incubate 16 – 44 hours at 37°C in a CO₂ incubator.

During this period make sure that the microplate is completely horizontal and do not agitate or move the microplate.

Day 3 Non aseptic Procedures

8. Empty the wells, rinse underside of the membrane with distilled water, wash the microplate five times with wash buffer and tap microplate on absorbent paper.
9. Dilute the biotinylated antibody 1:100 in dilution buffer and add 100 µl per well.
10. Incubate for one hour at room temperature.
11. Empty wells, rinse underside of the membrane with distilled water, wash the microplate five times with wash buffer and tap microplate on absorbent paper.
12. Dilute the streptavidin-poly-HRP 1:6,000 in dilution buffer and add 100 µl per well.
13. Incubate for one hour at room temperature.
14. Empty wells, rinse underside of the membrane with distilled water, wash the microplate five times with wash buffer and tap microplate on absorbent paper.
15. Add 50 µl substrate per well, incubate for 4-15 minutes at room temperature. Monitor spot intensity by eye.
16. Rinse underside of the membrane and wash the microplate with an excess of distilled water.
17. Dry the microplate and count the number of spots preferably using the A.EL.VIS automated spot analyzer.

Please inquire for information or demos of the A.EL.VIS spot analyzer systems