ELISpot Pro: Monkey IFN-γ (ALP)

Product Code: 3421M-2APT-2

CONTENTS:

Pre-coated plates, mAb MT126L (2 plates)

Detection mAb: 7-B6-1, ALP (150 µl)

BCIP/NBT-plus substrate (25 ml)

Anti-CD3 mAb (CD3-1) (100 µl)

STORAGE:
Shipped at ambient temperature. On arrival all reagents should be stored refrigerated at 4-8°C. Plates may be kept at room temperature. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use. Detection mAb is supplied in 0.1M Tris buffer with 0.002% Kathon CG. CD3-1 is supplied in sterile filtered (0.2 µm) PBS. Vials have been overfilled to ensure recovery of stated quantity.

Watch video tutorial

Guidelines for ELISpot Pro: Monkey IFN-γ (ALP)

Please read through before starting the assay

A Preparation and blocking of plate

1. Remove the plate from the sealed package and wash 4 times with sterile PBS (200 µl/well).
2. Condition the plate with medium containing 10% of the same serum as used for the cell suspensions (200 µl/well). Incubate for at least 30 minutes at room temperature.

B Incubation of cells in plate

1. Remove the medium and add the stimuli followed by the cell suspension. Alternatively cells and stimuli can be mixed before addition to the plate.
2. Put the plate in a 37°C humidified incubator with 5% CO_2 and incubate for 12-48 hours. Do not move the plate during this time and take measures to avoid evaporation (e.g. by wrapping the plate in aluminium foil).

C Detection of spots

1. Remove the cells by emptying the plate and wash 5 times with PBS, 200 µl/well.
2. Dilute the 7-B6-ALP 1:200 in filtered PBS containing 0.5% fetal calf serum. Add 100 µl to each well and incubate for 2 hours at room temperature.
3. Wash the plate 5 times with PBS, 200 µl/well.
4. Filter the ready-to-use substrate solution (BCIP/NBT-plus) through a 0.45 µm filter and add 100 µl of substrate per well. Develop until distinct spots emerge. Stop the colour development by washing extensively in tap water. If desirable, remove the underdrain (the soft plastic under the plate) and rinse the underside of the membrane.
5. Leave the plate to dry. Inspect and count spots in an ELISpot reader or in a dissection microscope.
6. Store plate in the dark at room temperature.

Hints and comments

These suggestions are based on the detection of antigen-specific immune responses using PBMC. If using T-cell clones, mixtures of separated cell fractions etc., other protocols may have to be considered.

Plate washing
Washing of plates can be done using a multi-channel micropipette. In washing steps not requiring sterile conditions (C1-C5), a regular ELISA plate washer can also be used, provided that the washing head is adapted to the ELISpot plates. Avoid getting liquid on the underside of the membrane as this may cause leakage due to capillary drainage.

Cells
Both freshly prepared and cryopreserved cells may be used in the assay. However it is recommended that the latter are rested for at least one hour to allow removal of cell debris before addition to the plate. Triplicates or duplicates of 250,000 cells per well are often used to assess antigen-specific responses. For polyclonal activators, the cell number may have to be reduced to avoid confluent spot formation. Protocols with other incubation times have to be established by the user.

Serum
The serum should be selected to support cell culture and give low background staining. We recommend the use of fetal calf serum. Alternatively serum-free medium evaluated for cell culture can be used.

Conjugate
To reduce unspecific background it is recommended to filter the detection mAb-enzyme conjugate (0.2 µm).

Assay controls
The number of cells responding to stimulation is often compared to the number of cells spontaneously producing the cytokine, which is determined by incubating the same number of cells in the absence of stimuli. A polyclonal activator such as phytohemagglutinin (1-10 µg/ml) is often used as a control for cell viability and functionality of the test system. The included anti-CD3 mAb can be used to induce cytokine secretion via TCR aggregation and is dependent on number, nature and viability of accessory cells. The antibody cross-reacts with CD3 on cynomolgus and rhesus macaque T cells. The recommended dilution is 1:1000 but should be titrated.

Buffers
PBS for washing and dilution should be filtered (0.2 µm) for optimal results. Avoid the inclusion of Tween or other detergents in the washing and incubation buffers.

Substrate development
Develop until distinct spots are visible in positive wells (usually 5-30 minutes). A general darkening of the membrane may occur but disappears after drying.

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