Monkey IFN-γ ELISpot^{PLUS}

Product Code: 3420M-4HPT-2

CONTENTS:

2 pre-coated plates, mAb GZ4

Vial 1 (yellow top)
Biotinylated detection mAb 7-B6-1 (40 µl)
Concentration 1 mg/ml

Vial 2 (white top)
Streptavidin-HRP (40 µl)

Vial 3 (black top)
Positive control anti-CD3 mAb CD3-1 (100 µl)

TMB substrate (25 ml)

The detection antibody is supplied in sterile filtered (0.2µm) PBS with 0.02% sodium azide. Streptavidin-HRP is supplied in PBS with 0.002% Kathon CG. Anti-CD3 mAb is supplied in sterile filtered (0.2 µm) PBS. Vials have been overfilled to ensure recovery of the specified amount.

STORAGE:

Shipped at ambient temperature. On arrival all reagents should be stored refrigerated at 4-8°C. Plates should be kept at room temperature.

Guidelines for Monkey IFN-γ ELISpot^{PLUS}

Please read through before starting the assay

A Preparation of ELISpot plate (sterile conditions)

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

B Incubation of cells in plate (sterile conditions)

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. The mAb CD3-1, included in the kit, is recommended as a positive control for cytokine production at a dilution of 1:1000.
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 detection antibody (7-B6-1-biotin) to 1 µg/ml in PBS containing 0.5% fetal calf serum (PBS-0.5% FCS). Add 100 µl/well and incubate for 2 hours at room temperature.
3. Wash plate as above (step C1).
4. Dilute the Streptavidin-HRP (1:1000) in PBS-0.5% FCS and add 100 µl/well. Incubate for 1 hour at room temperature.
   Please note that sodium azide used in buffers will inhibit HRP activity.
5. Wash plate as above (step C1).
6. Add 100 µl/well of the ready-to-use TMB substrate solution and develop until distinct spots emerge.
7. Stop color development by washing extensively in deionized water. If desirable, remove the underdrain (the soft plastic under the plate) and rinse the underside of the membrane.
8. Leave the plate to dry. Inspect and count spots in an ELISpot reader or in a dissection microscope.
9. 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 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.

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. Human serum is not recommended as it may contain heterophilic antibodies or intrinsic analyte which may interfere with the assay.

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 the included anti-CD3 mAb or phytohemagglutinin (1-10 µg/ml), is often used as a control for cell viability and functionality of the test system.

Detection antibody
To reduce unspecific background it is recommended to filter (0.2 µm) the working dilution of detection mAb.

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
Development is made until distinct spots are visible in positive wells (usually 5-30 minutes). A general darkening of the membrane may occur during development but disappears after drying. Preferably use deionized water to stop the plates since some ions may cause fading of TMB spots.

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