ELISpot^{PLUS} for Human MIP-1β

Product Code: 3495-2AW-Plus

CONTENTS:

Vial 1 (yellow top)
Monoclonal antibody MIP1β-I (600 µl)
Concentration: 1 mg/ml

Vial 2 (blue top)
Biotinylated monoclonal antibody MIP1β-II (50 µl)
Concentration: 0.5 mg/ml

Vial 3 (white top)
Streptavidin-Alkaline Phosphatase (50 µl)

To ensure total recovery of stated quantity, vials have been overfilled.

ELISpot plates (4 PVDF plates)

BCIP/NBT-plus substrate (2 x 25 ml)

STORAGE:
Shipped at ambient temperature. On arrival all reagents should be stored refrigerated at 4-8°C. Plates should be kept at room temperature. Antibodies are supplied in sterile filtered (0.2 µm) PBS with 0.02% sodium azide. Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.15% Kathon CG.

Guidelines for Human MIP-1β ELISpot^{PLUS}

Please read through before starting the assay

A Preparation of ELISpot plate (sterile conditions)

1. Dilute the coating antibody (MIP1β-I) to 15 µg/ml in sterile PBS, pH 7.4.
2. Remove the ELISpot plate (MAIPSWU included in this kit) from the package and pre-wet the membrane by adding 50 µl 70% ethanol per well. For these plates the maximum ethanol incubation time is 2 minutes.
3. Always remove the plate from the plate tray before manually emptying the plate. Wash plate 5 times with sterile water, 200 µl/well.
4. Add 100 µl/well of the antibody solution and incubate overnight at 4-8°C.

B Incubation of cells in plate (sterile conditions)

1. Remove excess antibody and wash plate 5 times with sterile PBS, 200 µl/well.
2. Add 200 µl/well with medium containing 10% of the same serum as used for the cell suspensions. Incubate for at least 30 minutes at room temperature.
3. Remove the medium and add the stimuli followed by the cell suspension. Alternatively cells and stimuli can be mixed before addition to the plate.
4. 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 (MIP1β-II-biotin) to 0.5 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-ALP (1:1000) in PBS-0.5% FCS and add 100 µl/well. Incubate for 1 hour at room temperature.
5. Wash plate as above (step C1).
6. Filter the ready-to-use substrate solution (BCIP/NBT-plus) through a 0.45 µm filter and add 100 µl/well. Develop until distinct spots emerge.
7. Stop colour development by washing extensively in tap water. Remove the plate from the tray 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

Please read through before starting the assay

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.

Plates

To obtain maximal antibody binding capacity, the plates need to first be activated by a brief treatment with ethanol. It is essential that the membrane is not allowed to dry after the treatment. If this occurs the treatment step (A2-3) needs to be repeated before adding the coating antibody.

Plate washing

Always remove the plate from the plate tray before manually emptying the plate. 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. MIP-1β can be spontaneously produced by monocytes and lower cell number or depletion of monocytes may be required for detection of specific responses. 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 LPS (1-10 ng/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. Although possible to use, we do not recommend 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 10-30 minutes). A general darkening of the membrane may occur but disappears after drying.

NOTE; for research use only.
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