ELISpot Plus:
Human IL-8 (ALP)

PRODUCT CODE: 3560-4APW-2

CONTENTS:

2 pre-coated plates, mAbs MT8H6/8F19

Vial 1
Detection mAb: 26E5, biotin (40 µl)
Concentration 0.5 mg/ml

Vial 2
Streptavidin-ALP (40 µl)

BCIP/NBT-plus substrate (25 ml)

The detection antibody is supplied in sterile filtered (0.2µm) PBS with 0.02% sodium azide. Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.002% Kathon CG. Vials have been over-filled to ensure recovery of the specified amount.

STORAGE:

Shipped at ambient temperature. On arrival all reagents should be stored 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.

Watch video tutorial

Guidelines for ELISpot Plus: Human IL-8 (ALP)

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. Add 200 µl/well of cell culture medium to block and condition the plate. 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.
2. Put the plate in a 37°C humidified incubator with 5% CO_2 and incubate for 18-48 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 (26E5-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 color 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.
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 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. Cell numbers need to be selected based on cell type and stimuli used. For LPS-stimulated human PBMC, around 1000-5000 cells/well is recommended.

Serum
For blocking and cell incubation, we recommend serum-free medium evaluated for cell culture. Use of medium supplemented with FCS may result in membrane background staining due to presence of bovine IL-8.

Assay controls
The number of cells responding to stimulation is often compared to the number of cells spontaneously producing cytokine which is determined by incubating the same number of cells in the absence of stimuli. LPS stimulation can be used as a control for cell viability and functionality of the assay.

Detection antibody
Diluted detection mAb can be filtered (0.2 µm) to reduce the risk of unspecific background.

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.

MABTECH

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