ELISpot Plus:
Human Perforin (ALP)

PRODUCT CODE: 3465-4APW-2

CONTENTS:

2 pre-coated plates, mAbs Pf-80/164

Vial 1
Detection mAb: Pf-344, biotin (40 µl)
Concentration 1 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.

Guidelines for ELISpot Plus: Human Perforin (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. 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.
2. Put the plate in a 37°C humidified incubator with 5% CO_2 and incubate for approximately 72 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 (Pf-344-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-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 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 analyte, which is determined by incubating the same number of cells in the absence of stimuli. The spontaneous production may be higher for perforin than for the IFN-γ system. Polyclonal activation with PHA (1-10 µg/ml) or PMA/ ionomycin (e.g. 20 ng/ml/ 1 µM) is often included as a control for cell viability and functionality of the test system.

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

Updated on 2025/02/18

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