ELISpot^{PLUS} for Human Perforin

Product Code: 3465-4APW-4

CONTENTS:
Precoated plates, mAb Pf-80/164
(4 white plates)

Vial 1 (blue top)
Biotinylated monoclonal antibody Pf-344 (50 µl)
Concentration: 1 mg/ml

Vial 2 (white top)
Streptavidin-Alkaline Phosphatase (50 µl)

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

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

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 (0.2µm) PBS with 0.02% sodium azide. Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.15% Kathon CG.

Guidelines for Human Perforin 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 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 (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 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 (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. Add 100 µl/well of substrate solution (e.g. BCIP/NBT) and develop until distinct spots emerge.
7. Stop 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.
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 human peripheral blood mononuclear cells (PBMC). If using T-cell clones, mixtures of separated cell fractions etc., other protocols may have to be considered.

Perforin system
Carefully follow the recommended protocol to achieve optimal and reliable results. The spots from perforin secreting cells will be small and distinct and are hard to detect without microscope. The perforin spots normally demands longer substrate development time than e.g. human IFN-γ spots.

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.

Assay controls

The number of cells responding to antigen stimulation is often compared to the number of cells spontaneously producing the cytokine. Spontaneous production is determined by incubating the same number of cells in the absence of antigen. The spontaneous production may be higher for perforin than for the human IFN-γ system. A polyclonal activator such as phytohemagglutinin (1-10 μg/ml) is often included as a control for cell viability and functionality of the test system.

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 seen in positive wells (usually 20-50 minutes). A general darkening of the membrane may occur during development but disappears after drying.

NOTE; for research use only.
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