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ELISpot^{PLUS} for Human Interferon-γ

Product Code: 3420-4HST-4

CONTENTS:

- ❑ Precoated strip plates, mAb 1-D1K
  (4 transparent plates)
- ❑ Empty plate frame for transfer of strips
- ❑ Vial 1 (yellow top)
  Biotinylated monoclonal antibody 7-B6-1 (50 µl)
  Concentration: 1 mg/ml
- ❑ Vial 2 (white top)
  Streptavidin-Horseradish Peroxidase (50 µl)
- ❑ Vial 3 (black top)
  Positive control anti-CD3 mAb CD3-2 (100 µl)
- ❑ TMB substrate (2 x 25 ml)

STORAGE:
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-HRP is supplied in PBS with 0.15% Kathon CG®.

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

Guidelines for Human IFN-γ ELISpot^{PLUS}

A Preparation and blocking of precoated plate *(sterile conditions)*

1. Assemble the required number of strips in the extra plate frame and wash 4 times with sterile PBS (200 µl/well). Seal the bag with the remaining strips and store at room temperature.
2. Block with medium containing 10% of the same serum as used for the cell suspensions (200 µl/well). Incubate for ≥30 minutes at room temperature.

B Incubation of cells in plate *(sterile conditions)*

1. Remove the medium and add the cell suspension including possible stimulatory agents such as antigen (final volume 100-150 µl/well). The mAb CD3-2, included in the kit, is recommended as a positive control for cytokine production using a final concentration of 100 ng/ml.
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 wrap the plate in aluminium foil to avoid evaporation.

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 colour development by washing extensively in tap water. Remove the underdrain (the soft plastic under the plate) and rinse the back of the membrane.
8. Leave the plate to dry. Inspect and count spots in a dissection microscope (x40) or in an ELISpot reader.
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 human PBMC. If using T-cell 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 with good results. 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 in order to avoid confluent spot formation. A serum that is good for cell culture and gives low background staining should be chosen; fetal calf serum is normally recommended.

Cell incubation time
Antigen-specific stimulation of cells can result in detectable spots after 12 hours of incubation. If desired, the incubation time can be extended up to 48 hours. Protocols with shorter incubation times have to be developed and evaluated 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. 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.

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
Development is made until distinct spots are seen in positive wells (usually 20-40 minutes). A general darkening of the membrane may occur during development but disappears after drying.

For further questions on the kit or protocol, please contact Mabtech.