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ELISpot^{PRO} for Human Interferon-γ

Product Code: 3420-2AST-2

CONTENTS:

- ❑ Precoated strip plates, mAb 1-D1K (2 transparent plates)
- ❑ Empty plate frame for transfer of strips
- ❑ 7-B6-1-ALP conjugate (150 µl)
- ❑ BCIP/NBT-plus substrate (25 ml)
- ❑ Positive control anti-CD3 mAb CD3-2 (100 µl)

STORAGE:
All reagents should be stored refrigerated at 4–8°C. Plates may be kept at room temperature. Antibodies are supplied in sterile filtered (0.2 µm) PBS with 0.02% sodium azide. Conjugate is supplied in 0.1M Tris buffer with 0.15% Kathon CG®.

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

Guidelines for Human IFN-γ ELISpot^{PRO}
(precoated plates and one-step detection reagent)

A Preparation and blocking of precoated plate

1. Assemble the required number of strips in the extra plate frame and wash 4 times with sterile PBS (200µl/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

1. Remove the blocking medium and add the cell suspension including possible stimulatory agents such as antigen (final volume of 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 one-step detection reagent (alkaline phosphatase-conjugated detection mAb 7-B6-1) 1:200 in filtered PBS containing 0.5% fetal calf serum. Add 100 µl to each well. Incubate for 2 hours at room temperature.
3. Wash the plate 5 times with PBS, 200 µl/well.
4. Filter the ready-to-use substrate solution (BCIP/NBT-plus) through a 0.45 µm filter and add 100 µl of substrate per well. Develop until spots emerge; longer development than 15 min can result in background staining. Stop the colour development by washing extensively in tap water. Remove the underdrain (the soft plastic under the plate) and rinse the back of the membranes.
5. Leave the plate to dry. Inspect and count spots in a dissection microscope (x40) or in an ELISpot reader.
6. Store plate in the dark at room temperature.

Hints and comments to the use of ELISpot^{PRO}

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.

Blocking step
The blocking step (A2) can be performed whilst preparing the cell suspensions. Blocking time can be prolonged for up to five hours.

Plate washing
For washing with sterile PBS (A1) a multi-channel micropipette can be used. For washing of plates under non-sterile conditions (C1-C3), PBS can be added by pipetting or by use of a squirting bottle. A regular ELISA plate washer can be used provided the washing head is adapted to the ELISpot plates. A vacuum manifold should not be used for washing of precoated plates.

Cells
Both freshly prepared and cryopreserved cells may be used in the assay with good results. Triplicates of 250,000 cells per well are often used to assess antigen-specific responses of PBMC. For polyclonal activators, the cell number may have to be decreased 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 PBMC 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.

Substrate development
Mabtech's recommendation for the duration of the substrate development reaction is to not exceed 15 minutes of development. Longer development can result in background staining that interferes with the subsequent analysis of spots.

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