ELISpot Pro:
Human IFN-γ (HRP)

Product Code: 3420-2HST-2

CONTENTS:

Pre-coated strip plates, mAb 1-D1K (2 clear plates)
Empty plate frame for transfer of strips

Detection mAb: 7-B6-1, HRP (150 µl)

TMB substrate (25 ml)

Positive control: Anti-CD3 mAb (CD3-2) (100 µl)

STORAGE:
Shipped at ambient temperature. On arrival all reagents should be stored refrigerated 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. Detection mAb is supplied in storage buffer with stabilizing bovine protein and 0.002% Kathon CG. CD3-2 is supplied in sterile filtered (0.2 µm) PBS. Vials have been overfilled to ensure recovery of stated quantity.

Watch video tutorial

Guidelines for ELISpot Pro: Human IFN-γ (HRP)

Please read through before starting the assay

A Preparation and blocking of 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. 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

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. The mAb CD3-2, included in the kit, is recommended as a positive control for cytokine production in a dilution of 1:1000.
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 7-B6-HRP (horseradish peroxidase-conjugated detection mAb 7-B6-1) 1:200 in filtered PBS containing 0.5% fetal calf serum. Add 100 µl to each well and incubate for 2 hours at room temperature.
   Please note that sodium azide used in buffers will inhibit HRP activity.
3. Wash the plate 5 times with PBS, 200 µl/well.
4. Add 100 µl/well of the ready-to-use TMB substrate solution and develop until distinct spots emerge. Stop the colour development by washing extensively in deionized water. Remove the plate frame from the plastic tray and rinse the underside of the membrane.
5. Leave the plate to dry. Inspect and count spots in an ELISpot reader or in a dissection microscope.
6. 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 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. Avoid getting liquid on the underside of the membrane as this may cause leakage due to capillary drainage.

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.

Conjugate
To reduce unspecific background it is recommended to filter the detection mAb-enzyme conjugate (0.2 µm).

Assay controls
The number of cells responding to stimulation is often compared to the number of cells spontaneously producing the cytokine, which is determined by incubating the same number of cells in the absence of stimuli. A polyclonal activator such as the included anti-CD3 mAb, CD3-2 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. Avoid the inclusion of Tween or other detergents in the washing and incubation buffers.

Substrate development
Develop until distinct spots are visible in positive wells (usually 2-20 minutes). A general darkening of the membrane may occur but disappears after drying. Preferably use deionized water to stop the plates since some ions may cause fading of TMB spots.

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Updated on 2025/09/25

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