ELISpot Path: Vaccinia Human IFN-γ

PRODUCT CODE: 3420-4AST-P7-1

CONTENTS:

1 pre-coated strip plate (mAb 1-D1K) and empty plate frame

Detection mAb: 7-B6-1, biotin, 1 mg/ml (40 µl)

Streptavidin-ALP (40 µl)

PepPool: Vaccinia (CD4), human (1 vial)
PepPool: Vaccinia (CD8), human (1 vial)

Positive control: Anti-CD3 mAb (CD3-2) (100 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. Anti-CD3 mAb is supplied in sterile filtered (0.2 µm) PBS. Vials have been overfilled to ensure recovery of the specified amount.

STORAGE:

Shipped at ambient temperature. On arrival all reagents should be stored at 4-8 °C except the peptide pools that should be stored frozen at -20 °C or below. Plates may be kept at room temperature. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

Watch video tutorial

Guidelines

PLEASE READ THROUGH BEFORE STARTING THE ASSAY

A Preparation of ELISpot 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. 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. The peptide pools can be used separately or mixed. If used separately, dissolve the peptide pools by addition of 40 µl DMSO to each vial, then add 85 µl PBS. If used mixed, add 40 µl DMSO to one vial and transfer the solution to the second vial. Add 85 µl PBS to the first vial, to rinse the vial, and then transfer the solution to the second vial. The concentrations of these stock solutions are 200 µg/ml of each peptide. Aliquot the pools and store at -20°C or below.
   Empty the plate and add 50 µl/well of peptides or controls, followed by 50 µl cell suspension/well. Alternatively, mix cells and stimuli before addition of 100 µl/well. The final peptide concentration should be 2 µg/ml of each peptide. Anti-CD3 is recommended as a positive control at a dilution of 1:1000.
2. Put the plate in a 37°C humidified incubator with 5% CO_2 and incubate for 18-48 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 (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-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. Remove the plate frame from the plastic tray 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 250,000-500,000 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.

Anti-CD28 mAb provides a co-stimulatory signal to antigen-specific responses by binding to CD28 on T cells. Addition of an anti-CD28 mAb together with antigen (step B1) can be used to enhance antigen-specific responses. However, if the concentration of anti-CD28 mAb is too high, non-specific cytokine secretion may be elevated.

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 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.

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.

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Mabtech AB (Head Office)
Sweden
Tel: +46 8 716 27 00
mabtech@mabtech.com

Mabtech, Inc.
USA
Tel: +1 513 871-4500
mabtech.usa@mabtech.com

www.mabtech.com