

ELISpot^{PRO} for Human IFN- γ

Product Code: 3420-2APW-2

CONTENTS:

Pre-coated plates, mAb 1-D1K (2 white plates)

7-B6-1-ALP conjugate (150 μ l)

BCIP/NBT-plus substrate (25 ml)

Positive control anti-CD3 mAb CD3-2 (100 μ l)

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 may be kept at room temperature. Conjugate is supplied in 0.1M Tris buffer with 0.15% Kathon CG. CD3-2 is supplied in sterile filtered (0.2 μ m) PBS.

Guidelines for Human IFN- γ ELISpot^{PRO}

Please read through before starting the assay

A Preparation and blocking of plate

1. Remove the plate from the sealed package and wash 4 times with sterile PBS (200 μ l/well).
2. Condition the plate with medium containing 10% of the same serum as used for the cell suspensions (200 μ l/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% CO₂ 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 μ l/well.
2. Dilute the 7-B6-ALP 1:200 in filtered PBS containing 0.5% fetal calf serum. Add 100 μ l to each well and incubate for 2 hours at room temperature.
3. Wash the plate 5 times with PBS, 200 μ l/well.
4. Filter the ready-to-use substrate solution (BCIP/NBT-plus) through a 0.45 μ m filter and add 100 μ l of substrate per well. Develop until distinct spots emerge. Stop the 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.
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

Please read through before starting the assay

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 $\mu\text{g}/\text{ml}$) 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 10-30 minutes). A general darkening of the membrane may occur but disappears after drying.

NOTE; for research use only.

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MABTECH AB
Box 1233
SE-131 28 Nacka Strand
Sweden
Tel: +46 8 716 27 00
Fax: +46 8 716 27 01
E-mail: mabtech@mabtech.com
www.mabtech.com

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the following standards:



MABTECH Inc
M.E.B. 220
3814 West Street
Cincinnati, OH 45227
USA
Tel: +1 513 871 4500
Fax: +1 513 871 7353
E-mail: mabtech.usa@mabtech.com

MABTECH AUSTRALIA Pty Ltd
resolvingIMAGES
Unit 22, 196 Settlement Road
Thomastown Victoria 3074
Australia
Tel: +61 3 9466 4007
Fax: +61 3 9466 4003
E-mail: mabtech.au@mabtech.com

MABTECH AB Büro Deutschland
Germany
Tel: +49 40 4135 7935
Fax: +49 40 4135 7945
E-mail: mabtech.de@mabtech.com

MABTECH AB Bureau de liaison France
BP 255, 1300 route des Crêtes
06905 Sophia Antipolis
France
Tel: +33 (0)4 92 38 80 70
Fax: +33 (0)4 92 38 80 71
E-mail: mabtech.fr@mabtech.com

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