

# ELISpot<sup>PLUS</sup> for Human IFN- $\gamma$

Product Code: 3420-4HST-4

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## 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  $\mu$ l)

Concentration: 1 mg/ml

### **Vial 2 (white top)**

Streptavidin-Horseradish Peroxidase (50  $\mu$ l)

### **Vial 3 (black top)**

Positive control anti-CD3 mAb CD3-2 (100  $\mu$ l)

**TMB substrate (2 x 25 ml)**

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 should be kept at room temperature. Antibodies are supplied in sterile filtered (0.2 $\mu$ m) PBS with 0.02% sodium azide. Streptavidin-HRP is supplied in PBS with 0.15% Kathon CG.

# Guidelines for Human IFN- $\gamma$ ELISpot<sup>PLUS</sup>

Please read through before starting the assay

## **A Preparation of ELISpot plate (sterile conditions)**

1. Remove the plate from the sealed package and wash 4 times with sterile PBS (200  $\mu$ l/well).
2. Condition the plate with medium containing 10% of the same serum as used for the cell suspensions (200  $\mu$ l/well). Incubate for at least 30 minutes at room temperature.

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

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<sub>2</sub> 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  $\mu$ l/well.
2. Dilute the detection antibody (7-B6-1-biotin) to 1  $\mu$ g/ml in PBS containing 0.5% fetal calf serum (PBS-0.5% FCS). Add 100  $\mu$ l/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  $\mu$ l/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  $\mu$ l/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.
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

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.

## **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.

## **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**

Development is made until distinct spots are seen in positive wells (usually 10-30 minutes). A general darkening of the membrane may occur during development 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](mailto:mabtech@mabtech.com)  
[www.mabtech.com](http://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](mailto:mabtech.usa@mabtech.com)

MABTECH AUSTRALIA Pty Ltd  
44 Gresswell Road  
Macleod, VIC 3085  
Australia  
Tel: +61 3 9459 9630  
Fax: +61 3 9455 0084  
E-mail: [mabtech.au@mabtech.com](mailto: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](mailto: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](mailto:mabtech.fr@mabtech.com)

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