

# ELISpot<sup>PLUS</sup> for Human IgE

Product Code: 3810-2AW-Plus

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## CONTENTS:

### **Vial 1 (green top)**

Monoclonal antibody 107 (600 µl). Concentration: 1 mg/ml

### **Vial 2 (purple top)**

Biotinylated monoclonal antibodies 107/182/101, a combination of three different antibodies (50 µl). Concentration: 1 mg/ml

### **Vial 3 (white top)**

Streptavidin-Alkaline Phosphatase (50 µl)

### **Vial 4 (black top)**

B-cell activator: anti-CD40 (mAb S2C6) (100 µl). Concentration: 1 mg/ml

Grey plastic box:

Lyophilised recombinant human IL-4 (2 x 1 µg)

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

### **ELISpot plates (4 PVDF plates)**

### **BCIP/NBT substrate (2 x 25 ml)**

## STORAGE:

Shipped at ambient temperature. On arrival antibodies, conjugate and substrate should be stored refrigerated at 4-8°C. Plates may be kept at room temperature. The grey plastic box should be stored frozen at -20°C. Antibodies are supplied in sterile filtered (0.2µm) PBS with 0.02% sodium azide, except mAb S2C6 that is supplied without azide. Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.15% Kathon CG.

# Guidelines for Human IgE ELISpot<sup>PLUS</sup>

Please read through before starting the assay

## A Preparation of ELISpot plate (sterile conditions)

1. Dilute the coating antibody (107) to 15 µg/ml in sterile PBS, pH 7.4.
2. Remove the ELISpot plate (MAIPSWU included in this kit) from the package and pre-wet the membrane by adding 50 µl 70% ethanol per well. For these plates the maximum ethanol incubation time is 2 minutes.
3. Always remove the plate from the plate tray before manually emptying the plate. Wash plate 5 times with sterile water, 200 µl/well.
4. Add 100 µl/well of the antibody solution and incubate overnight at 4-8°C.

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

1. Remove excess antibody and wash plate 5 times with sterile PBS, 200 µl/well.
2. Add 200 µl/well with medium containing 10% of the same serum as used for the cell suspensions. Incubate for at least 30 minutes at room temperature.
3. Stimulate the cells with 1 µg/ml anti-CD40 (mAb S2C6) and 30 ng/ml rhIL-4 in separate tubes/plates for approx. 5 days. Reconstitute one vial of rhIL-4 in 1 ml PBS to obtain 1 µg/ml. Leave for 15 min and then vortex. Use directly or store in aliquots at -20°C for future use. After stimulation, wash the cells to ensure removal of any secreted antibodies. Resuspend the cells in medium and add the cell suspension to the ELISpot plate (after removal of the blocking medium).
4. Put the plate in a 37°C humidified incubator with 5% CO<sub>2</sub> and incubate for 16-24 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 antibodies (107/182/101-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 colour development by washing extensively in tap water. Remove the plate from the 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

## Please read through before starting the assay

These suggestions are based on the detection of antigen-specific immune responses using PBMC. If using cell clones, mixtures of separated cell fractions etc., other protocols may have to be considered.

### **Plates**

To obtain maximal antibody binding capacity, the plates need to first be activated by a brief treatment with ethanol. It is essential that the membrane is not allowed to dry after the treatment. If this occurs the treatment step (A2-3) needs to be repeated before adding the coating antibody.

### **Plate washing**

Always remove the plate from the plate tray before manually emptying the plate. 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 100,000-300,000 cells per well are recommended but may need to be modified depending on the expected frequencies. 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.

### **Assay controls**

The number of cells responding to stimulation is often compared to the number of cells spontaneously producing IgE, which is determined by incubating the same number of cells in the absence of stimuli. The polyclonal activator supplied can be used as a control for cell viability and functionality of the test system.

### **Detection antibody**

To reduce unspecific background it is recommended to filter (0.2  $\mu$ m) the working dilution of detection mAb.

### **Buffers**

PBS for washing and dilution should be filtered (0.2  $\mu$ m) for optimal results. Although possible to use, we do not recommend 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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