ELISpot^{PLUS} for Human IgE

Product Code: 3810-2HW-Plus

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-Horseradish Peroxidase (50 µl)

Vial 4 (black top)
B-cell activator: anti-CD40 (mAb S2C6) (100 µl). Concentration: 1 mg/ml
Box 2, inside box 1:
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)

TMB 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-HRP is supplied in 0.1 M Tris buffer with 0.15% Kathon CG.

Guidelines for Human IgE ELISpot^{PLUS}

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_2 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-HRP (1:1000) in PBS-0.5% FCS and add 100 µl/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 µl/well of ready-to-use TMB substrate solution and 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 μm) the working dilution of detection mAb.

Buffers

PBS for washing and dilution should be filtered (0.2 μm) 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. Use water of good quality to stop since poor quality may cause fading of TMB spots.

NOTE; for research use only.
MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages therefrom.

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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