ELISpot^{PLUS} for Monkey IL-2

Product Code: 3440M-4APT-4

CONTENTS:
Precoated plates, mAbs IL2M-I/249 (4 transparent plates)

Vial 1 (yellow top)
Biotinylated monoclonal antibody IL2-II (50 µl)
Concentration: 0.5 mg/ml

Vial 2 (white top)
Streptavidin-Alkaline Phosphatase (50 µl)

Vial 3 (black top)
Positive control anti-CD3 mAb CD3-1 (100 µl)

BCIP/NBT-plus 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 (0.2µm) PBS with 0.02% sodium azide. Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.15% Kathon CG.

Guidelines for Monkey IL-2 ELISpot^{PLUS}

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 µl/well).
2. Condition the plate with medium containing 10% of the same serum as used for the cell suspensions (200 µl/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 CD3-1, included in the kit, is recommended as a positive control for cytokine production in a dilution of 1:1000. (It has been tested for rhesus and cynomolgus macaques but not for other monkey species).
2. Put the plate in a 37°C humidified incubator with 5% CO_2 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 µl/well.
2. Dilute the detection antibody (IL2-II-biotin) to 0.5 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. If desirable, remove the underdrain (the soft plastic under the plate) 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 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 antigen stimulation is often compared to the number of cells spontaneously producing the cytokine. Spontaneous production is determined by incubating the same number of cells in the absence of antigen. A polyclonal activator, such as the included anti-CD3 mAb 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.
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

2013-01-23