ELISpot for Rat IFN-γ

Product Code: 3220-2H

CONTENTS, kit for 4 plates:

Vial 1 (green top)
Monoclonal antibody rIFNγ-I (600 µl)
Concentration: 1 mg/ml

Vial 2 (yellow top)
Biotinylated monoclonal antibody rIFNγ-II (50 µl)
Concentration: 1 mg/ml

Vial 3 (white top)
Streptavidin-Horseradish Peroxidase (500 µl)

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. Antibodies are supplied in sterile filtered (0.2 µm) PBS with 0.02% sodium azide. Streptavidin-HRP is supplied in PBS with 0.15% Kathon CG.

Guidelines for Rat IFN-γ ELISpot

Please read through before starting the assay

A Preparation of ELISpot plate (sterile conditions)

1. Dilute the coating antibody (rIFNγ-I) to 15 µg/ml in sterile PBS, pH 7.4.
2. Remove the ELISpot plate from the package and if using a PVDF plate, pre-wet the membrane by adding ethanol. PVDF-plates from Millipore Corp., MAIPSWU, should be treated with 50 µl 70% ethanol per well for 2 minutes. PVDF-plates, type MSIP, should be treated with 15 µl 35% ethanol per well for maximum 1 minute.
3. 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 of medium containing 10% of the same serum as used for the cell suspensions. Incubate for at least 30 minutes at room temperature.
3. Remove the medium and add the cell suspension including possible stimulatory agents such as antigen.
4. 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 (rIFNγ-II-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 as above (step C1).
4. Dilute the Streptavidin-HRP* in PBS-0.5% FCS and add 100 µl/well. Incubate for 1 hour at room temperature.
   *Please, note that the peroxidase conjugate may require different dilutions depending on the choice of substrate in the final step. Thus, for AEC a dilution of 1 in 100 is usually suitable whereas a higher dilution (1 in 500-1000) may be required if using other substrates (e.g TMB). Please, also note that HRP-conjugates should not be used with buffers containing sodium azide as this compound will inhibit enzyme activity.
5. Wash as above (step C1).
6. Add 100 µl/well of substrate solution (e.g. TMB) and develop until distinct spots emerge.
7. Stop colour development by washing extensively in tap water. If desirable, remove the plate from the tray or the underdrain 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 spleen cells. If using T-cell clones, mixtures of separated cell fractions etc., other protocols may have to be considered.

Plates

We recommend the use of PVDF-based membrane plates. Maximal antibody binding capacity of these plates is obtained 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 MAIPSWU 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. 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.

Detection antibody

To reduce unspecific background it is recommended to filter (0.2 μm) the working dilution of detection mAb.

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 concanavalin A (1-10 μg/ml) is often included 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. 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
44 Gresswell Road
Macleod, VIC 3085
Australia
Tel: +61 3 9459 9630
Fax: +61 3 9455 0084
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

2010-10-12