Human TNF-α/GM-CSF FluoroSpot kit

Product Code: FS-0915-2

CONTENTS:

- Monoclonal antibody TNF3/4 (600 µl) red top
  Concentration: 0.5 mg/ml
- Monoclonal antibody 21C11 (300 µl) blue top
  Concentration: 1 mg/ml
- Monoclonal antibody TNF5-BAM (120 µl) blue top
- Biotinylated monoclonal antibody 23B6 (50 µl) green top
  Concentration: 1 mg/ml
- Monoclonal antibody anti-BAM-490 (120 µl)
- SA-550 (120 µl)
- LPS (120 µl)
  Concentration: 0.05 mg/ml
- Fluorospot plates (2 IPFL plates)
- Fluorescence enhancer-II (25 ml)

TNF3/4, 21C11 and 23B6 are supplied in sterile filtered (0.2 µm) PBS with 0.02% sodium azide. TNF5-BAM, SA-550, anti-BAM-490 and Fluorescence enhancer-II contain 0.002% Kathon CG.

STORAGE:
Shipped at ambient temperature. On arrival all reagents except LPS (in the grey box) should be stored refrigerated at 4-8°C. LPS should be stored at -20°C.

Guidelines for Human TNF-α/GM-CSF FluoroSpot

The protocol describes double staining for the detection of human TNF-α and GM-CSF. The protocol may also be adjusted to single staining. IPFL plates are coated with a mixture of mAbs: TNF3/4 for TNF-α and 21C11 for GM-CSF. Cells +/- stimuli are added and secreted TNF-α and GM-CSF will be captured by the specific mAbs. After cell removal, spots are detected in two steps. First, a mixture of TNF5-BAM (TNF-α) and 23B6-biotin (GM-CSF) is added, then a mixture of anti-BAM-490 (TNF-α) and SA-550 (GM-CSF).

A Preparation of plate (sterile conditions)

1. In the same tube, dilute the coating antibodies TNF3/4 to 15 µg/ml and 21C11 to 15 µg/ml in sterile PBS, pH 7.4. Filter the antibody solution using a 0.2 µm, low protein binding filter.
2. Pre-wet the plate membrane by treatment with 35% ethanol, 15 µl/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 to block/condition the membrane.
3. Remove the medium and add the stimuli followed by the cell suspension. Alternatively, cells and stimuli can be mixed before addition to the plate.
4. Put the plate in a 37°C humidified incubator with 5% CO_2 and incubate over night. 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. In the same tube, dilute the detection antibodies TNF5-BAM 1:200 and 23B6-biotin to 2 µg/ml in PBS containing 0.1% bovine serum albumin (PBS-0.1% BSA). Filter the antibody solution using a 0.2 µm, low protein binding filter. Add 100 µl/well and incubate for 2 hours at room temperature.
3. Wash as above (step C1).
4. In the same tube, dilute the anti-BAM-490 1:200 and SA-550 1:200 in PBS-0.1% BSA. Filter the solution using a 0.2 µm, low protein binding filter and add 100 µl/well. Incubate for 1 hour at room temperature. From this step on, cover the plate to limit light exposure.
5. Wash as above (step C1).
6. Empty the plate and add 50 µl/well of Fluorescence enhancer-II and leave the plate for 15 minutes at room temperature.
7. Empty the plate and remove residual Fluorescence enhancer-II by tapping the plate against clean paper towels.
8. Remove the underdrain (the soft plastic under the plate). Leave the plate in the dark to dry; plate should be completely dry before analysis. Inspect and count spots in a FluoroSpot reader. Store plate in the dark at room temperature.

Hints and comments

The assay is recommended for the analysis of macrophages, monocytes and dendritic cells in purified cell preparations or PBMC. Cells can be stimulated with e.g. LPS or other TLR ligands. These kits have not been evaluated for T cells.

Plates
The IPFL plates included in the kit have a low fluorescent PVDF-based membrane. 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 antibodies. The underdrain can be left on the plate all along, but then plates require a longer drying time before spots can be counted (step C8).

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/FluoroSpot plates.

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.

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. Cell numbers need to be selected based on cell type and stimuli used. For LPS-stimulated human PBMC, around 5000 cells/well is recommended. For purified LPS-stimulated monocytes, fewer cells will be required. 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 secreting cytokine which is determined by incubating the same number of cells in the absence of stimuli. LPS (included in the kit) can be used as a control for cell viability and functionality of the assay. For human PBMC it is recommended to use 50 ng/ml of the included LPS to 5000 cells/well.

Buffers
PBS for washing and dilution should be filtered (0.2 μm) for optimal results. We do not recommend the inclusion of Tween or other detergents in the washing and incubation buffers.

Detection reagents
To reduce unspecific background it is recommended to filter the working dilution of detection reagents. Use a 0.2 μm, low protein binding filter.

Analysis
We recommend the use of an automated FluoroSpot reader equipped with filters for FITC (excitation 490 nm/emission 510 nm) and Cy3 (excitation 550 nm/emission 570 nm). Filters should have high specificity to avoid bleed-through artifacts. Spots identified with FITC filter represent TNF-α producing cells and spots identified with Cy3 filter represent GM-CSF producing cells. Double producing cells are preferentially identified by a computerised overlay of TNF-α and GM-CSF spots. Fluorescent spots may fade due to excessive exposure to light and it is recommended to analyse the plate within one week of development.

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

2014-10-22