FluoroSpot Flex: Mouse IgM/IgG/IgA

Product Code: FS-60G49R50M-1

CONTENTS:

- Capture mAbs: Anti-Ig κ λ mAbs (MT24/JC5-1)
  800 µl
  Concentration: 0.5 mg/ml
- Detection mAb: Anti-IgM mAb (MT9A2), 490
  25 µl
- Detection mAbs: Anti-IgG mAbs (MG1/MG2a/MTG2c/MG2b/MG3), 550
  25 µl
- Detection mAb: Anti-IgA mAb (MT39A), 640
  25 µl
- Polyclonal activators:
  R848, 100 µl
  Lyophilized recombinant mouse IL-2, 0.5 µg
- FluoroSpot enhancer, 25 ml
- FluoroSpot plate (1 IPFL plate)

Capture antibodies are supplied in sterile filtered (0.2 µm) PBS with 0.02% sodium azide. R848 is supplied in sterile filtered (0.2 µm) PBS with 2% DMSO. Fluorophore conjugated detection antibodies and FluoroSpot enhancer contain 0.002% Kathon CG.

STORAGE:
Shipped at ambient temperature. Store antibodies and FluoroSpot enhancer at 4-8°C upon reciept. R848 and IL-2 should be stored at -20°C. Plates may be kept at room temperature.

Guidelines for Mouse IgM/IgG/IgA FluoroSpot

This kit contains reagents for the detection of mouse IgM, IgG, and IgA secreting B cells. B cells secreting antigen-specific antibodies as well as B cells secreting antibodies irrespective of antigen specificity can be measured with this kit. For analysis of memory B cells, cells may need to be pre-activated (see Hints and comments).

Antigen-specific IgM/IgG/IgA FluoroSpot:
Antigen is coated on the FluoroSpot plate and antigen-specific IgM, IgG, and IgA secreted by B cells bind to the immobilized antigen. Spots are visualized after addition of fluorophore labeled antibodies.
Total IgM/IgG/IgA FluoroSpot
IgM, IgG, and IgA from secreting B cells are captured by anti-mouse Ig κ λ specific mAbs MT24/JC5-1. Spots are visualized using the same detection reagents as for antigen-specific B cells.

A Preparation of plate (sterile conditions)

1. **Antigen-specific IgM/IgG/IgA FluoroSpot:**
   Dilute the antigen to suitable concentration (e.g. 1-50 µg/ml) in sterile PBS, pH 7.4.
   Total IgM/IgG/IgA FluoroSpot:
   Dilute capture mAbs MT24/JC5-1 to 40 µg/ml.
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 antigen or 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 sterile 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 cell suspension to the plate. Memory B cells may need pre-activation in tubes prior to this; please see Hints and comments section.
4. Place the plate in a 37°C humidified incubator with 5% CO_2. Incubate 5-6 hours or overnight. Avoid evaporation, e.g., by wrapping the plate in aluminum foil. Do not stack or move plates during incubation.

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 1:500. Add 100 µl/well and incubate 2 hours at 20 °C or overnight at 4 °C. From this step on, cover the plate to limit light exposure.
3. Wash as above (step C1).
4. Empty the plate and add 50 µl/well of FluoroSpot enhancer and incubate the plate for 15 minutes at room temperature.
5. Wash as above (step C1).
6. Remove the underdrain (the soft plastic under the plate). Leave the plate in the dark to dry. Inspect and count spots in a FluoroSpot reader. Store plate in the dark at room temperature.

Hints and comments

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

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 C6).

Plate washing
Plates can be washed using a multi-channel micropipette. When sterile conditions are not required (protocol steps C1-C6), an ELISA plate washer can be used if the washer head position has been adapted to ELISpot plates. After each wash cycle, empty the plate and tap it gently against absorbent paper.

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. Do not use mouse serum.

Cells
Freshly prepared or cryopreserved cells can be used. After thawing and washing of cryopreserved cells, leave diluted in cell culture medium at 37 °C for at least one hour. Remove cell debris and count the cells. B cells that have been activated *in vivo*, for instance as a result of repeated immunizations, may be analysed directly in the FluoroSpot wells without prior stimulation. Memory B cells may require polyclonal stimulation before secreting detectable amounts of antibody. Immunoglobulin secretion can be induced by pre-activation of cells with R848 and IL-2. Stimulate cells with a mixture of R848 (1 µg/ml) and IL-2* (10 ng/ml) in tubes for 2-3 days. After pre-activation, wash the cells extensively to remove secreted antibodies. Triplicates with 50.000-250.000 cells/well are recommended, but the cell number may need to be modified depending on the expected spot frequencies. Other incubation times have to be established by the user. * Reconstitute IL-2 with 1 ml PBS to obtain 0.5 µg/ml. Leave for 15 min and then vortex. Use directly or store in aliquots at -20°C.

Assay controls
Determination of the number of cells secreting IgM, IgG and IgA independent of antigen specificity is recommended as a postive control for the assay. It is also valuable for establishing the proportion of antigen-specific B cells. It is recommended to include coating without antigen or with an irrelevant antigen as controls.

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 LED490, LED550 and LED640. IgM spots are analyzed with LED490 filter, IgG spots with LED550, and IgA spots with a LED640 filter. Fluorescent spots may fade due to excessive exposure to light and it is recommended to analyze the plate within one week of development.

MABTECH

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Mabtech AB (Head Office)
Sweden
Tel: +46 8 716 27 00
mabtech@mabtech.com

Mabtech, Inc.
USA
Tel: +1 513 871-4500
mabtech.usa@mabtech.com

www.mabtech.com