

# FluoroSpot kit for Monkey IgG/IgA

Product Code: FS-05R24G-2

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## CONTENTS:

- ▶ Monoclonal antibodies MT91/145, a combination of two different antibodies (600 µl) yellow top  
Concentration: 0.5 mg/ml
- ▶ Monoclonal antibody MT57 (600 µl) blue top  
Concentration: 0.5 mg/ml
- ▶ Monoclonal antibodies MT78/145-Red (50 µl)
- ▶ Anti-human IgA-Green (50 µl)
- ▶
- ▶ Polyclonal activator: R848 (100 µl)  
Concentration: 1 mg/ml
- ▶ Lyophilised recombinant human IL-2 (1 µg)
- ▶ Fluorospot plates (2 IPFL plates)
- ▶ Fluorescence enhancer (25 ml)

## STORAGE:

Shipped at ambient temperature. On arrival antibodies and Fluorescence enhancer should be stored refrigerated at 4-8°C. R848 and IL-2 should be stored at -20°C. Plates may be kept at room temperature. MT91/145 and MT57 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. MT78/145-Red, anti-human IgA-Green and Fluorescence enhancer contain 0.15% Kathon CG.

# Guidelines for Monkey IgG/IgA FluoroSpot

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

## Detection of antigen-specific IgG/IgA producing B cells

Antigen is coated on the FluoroSpot plate and antigen-specific IgG and IgA secreted by B cells bind to the immobilized antigen. Spots are detected after addition of fluorophore labeled mAbs MT78/145-Red for IgG and anti-human IgA-Green for IgA.

## Detection of total IgG/IgA-producing B cells

IgG/IgA from secreting B cells are captured by mAbs MT91/145 (for IgG) and MT57 (for IgA). Total IgG/IgA spots are detected by the addition of mAbs MT78/145-Red for IgG and anti-human IgA-Green for IgA, i.e. the same detection as for antigen-specific B cells.

### A Preparation of plate (sterile conditions)

#### 1. Antigen-specific IgG/IgA:

Dilute the antigen to suitable concentration (e.g. 1-50  $\mu\text{g/ml}$ ) in sterile PBS, pH 7.4.

#### Total IgG/IgA:

Dilute the coating mAbs MT91/145 to 15  $\mu\text{g/ml}$  and MT57 to 15  $\mu\text{g/ml}$ . The antibodies should be diluted in the same tube.

2. Pre-wet the plate membrane by treatment with 35% ethanol, 15  $\mu\text{l/well}$ , for maximum 1 minute.
3. Wash plate 5 times with sterile water, 200  $\mu\text{l/well}$ .
4. Add 100  $\mu\text{l/well}$  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  $\mu\text{l/well}$ .
2. Add 200  $\mu\text{l/well}$  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. Put the plate in a 37°C humidified incubator with 5% CO<sub>2</sub> and incubate 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  $\mu\text{l/well}$ .
2. In the same tube, dilute the detection antibodies MT78/145-Red and anti-human IgA-Green 1:500 in PBS containing 0.5% fetal calf serum. Add 100  $\mu\text{l/well}$  and incubate for 2 hours at room temperature. From this step on, cover the plate to limit light exposure.
3. Wash as above (step C1).
4. Empty the plate and add 50  $\mu\text{l/well}$  of Fluorescence enhancer and incubate the plate for 15 minutes at room temperature.
5. Empty the plate and remove residual Fluorescence enhancer by firmly tapping the plate against clean paper towels.
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.
- 7.
- 8.

# Hints and comments

These suggestions are based on the detection of antigen-specific immune responses using PBMC. 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 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 fresh and cryopreserved cells may be used with good results. 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 100,000-500,000 cells per well are often used to assess antigen-specific responses. For detection of total IgG/IgA, less cells (e.g. 50,000-75,000 cells per well) may be used to avoid confluent spot formation.

B cells that have been activated *in vivo*, for instance as a result of vaccination, may be analysed directly in the FluoroSpot wells without prior stimulation. Typically, cells secreting antigen-specific antibodies can be detected in the circulation 6 to 9 days after vaccination.

Memory B cells may require polyclonal stimulation before secreting detectable amounts of antibody. Pre-stimulation can be made with a mixture of R848 (1  $\mu\text{g}/\text{ml}$ ) and rhIL-2 (10  $\text{ng}/\text{ml}$ ) (both included in the kit) in separate tubes/plates for 48-72 hours. After pre-stimulation, wash the cells to ensure removal of any secreted antibodies. Resuspend the cells in medium and add the cell suspension to the FluoroSpot plate.

Reconstitution of rhIL-2: Add 1 ml PBS to obtain 1  $\mu\text{g}/\text{ml}$ . Leave at room temperature for 15 minutes and then vortex. Use immediately or store in aliquots at  $-20^{\circ}\text{C}$ .

## Assay controls

The number of cells secreting IgG and IgA independent of antigen specificity is recommended as a positive control for the assay. It is also valuable for establishing the proportion of antigen-specific B cells.

## Buffers

PBS for washing and dilution should be filtered (0.2  $\mu\text{m}$ ) for optimal results. We do not recommend the inclusion of Tween or other detergents in the washing and incubation buffers.

## Detection antibody

To reduce unspecific background it is recommended to filter (0.2  $\mu\text{m}$ ) the working dilution of detection mAb.

## Analysis

Plates should be completely dry before analysis. Single colour analysis can be made in a microscope equipped with filters for FITC and Cy-3 but we recommend the use of an automated FluoroSpot reader with these filters. Green spots represent IgA producing cells and red spots represent IgG producing cells. 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.**

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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](mailto:mabtech@mabtech.com)  
[www.mabtech.com](http://www.mabtech.com)

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](mailto:mabtech.usa@mabtech.com)

MABTECH AB Büro Deutschland  
Germany  
Tel: +49 40 4135 7935  
Fax: +49 40 4135 7945  
E-mail: [mabtech.de@mabtech.com](mailto:mabtech.de@mabtech.com)

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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](mailto:mabtech.au@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](mailto:mabtech.fr@mabtech.com)