FluoroSpot Plus:
Human IL-22/IFN-γ/IL-10/IL-17A

FSP-18010703-2
FSP-18010703-10

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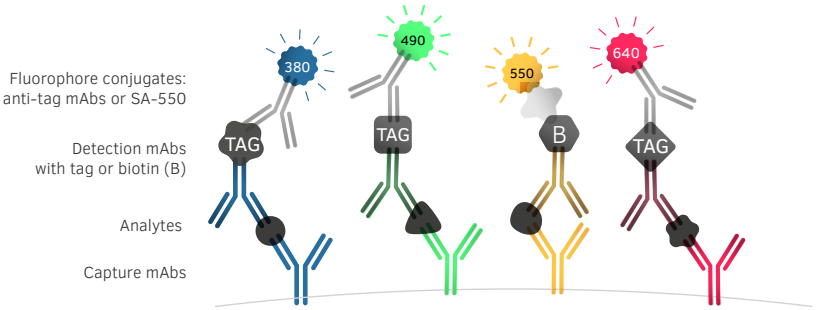
INTRODUCTION

FluoroSpot – General description

This four analyte FluoroSpot kit enables convenient enumeration of cells secreting either one, two, three or four analytes.

FluoroSpot combines the sensitivity of ELISpot with the capacity to analyze secretion of several analytes simultaneously. This highly sensitive cellular assay is robust, easy to perform, and suitable both for testing single samples and for large-scale screening.



Our FluoroSpot Plus kits include pre-coated plates ready to use. Monoclonal antibodies (mAbs) are coated onto a PVDF membrane in a 96-well plate. If several analytes are analyzed, mAbs with different specificities are coated. Detection of cells secreting single or multiple analytes is made possible using biotinylated and/or tag-labeled detection mAbs. The detection step is visualized and amplified by specific fluorophore-conjugated reagents and the resulting spots are analyzed in an automated reader.

CONTENTS AND STORAGE

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The kit is shipped at ambient temperature. All reagents should be stored at 4-8°C upon arrival. Plates should be stored at room temperature. The expiry date indicates how long unopened products, stored according to instructions, are recommended to remain in use.

	Component	Concentration	2-plate kit	10-plate kit
Detection antibodies	anti-IL-22 mAb MT7B27-DIG		120 µl	600 µl
	anti-IFN-γ mAb 7-B6-1-BAM		120 µl	600 µl
	anti-IL-10 mAb 12G8, biotinylated	1 mg/ml	50 µl	250 µl
	anti-IL-17A mAb MT504-WASP		120 µl	600 µl
Fluorophore conjugates	anti-DIG-380		120 µl	600 µl
	anti-BAM-490		120 µl	600 µl
	SA-550		120 µl	600 µl
	anti-WASP-640		120 µl	600 µl
Co-stimulator	anti-CD28 mAb CD28A	0.1 mg/ml	100 µl	500 µl
Positive control	anti-CD3 mAb CD3-2	0.1 mg/ml	100 µl	100 µl
Fluorescence enhancer	Ready-to-use Fluorescence enhancer-II		25 ml	60 ml
Pre-coated plates	Plates pre-coated with: mAbs MT12A3, 1-D1K, 9D7 and MT44.6		2	10

Anti-CD28 and anti-CD3 mAbs are supplied in sterile filtered (0.2 µm) PBS. The biotinylated antibody contains 0.02% sodium azide. The DIG-, BAM-, and WASP-conjugated antibodies, fluorophore-conjugated reagents, and the Fluorescence enhancer contain 0.002 % Kathon CG. Vials have been overfilled to ensure recovery of stated volume.

ASSAY PROTOCOL

Please consult the table below for recommendations on dilutions and cell incubation time. The reagents should not be diluted in advance. For additional information, read the Tips For A Successful Assay section.

	Component	Dilution	Step
Detection antibodies	anti-IL-22 mAb MT7B27-DIG	1:200	**C2**
	anti-IFN-γ mAb 7-B6-1-BAM	1:200	**C2**
	anti-IL-10 mAb 12G8, biotinylated	1:500	**C2**
	anti-IL-17A mAb MT504-WASP	1:200	**C2**
Fluorophore conjugates	anti-DIG-380	1:200	**C4**
	anti-BAM-490	1:200	**C4**
	SA-550	1:200	**C4**
	anti-WASP-640	1:200	**C4**
Co-stimulator	anti-CD28 mAb CD28A	1:1000	**B1**
Positive control	anti-CD3 mAb CD3-2	1:1000	**B1**
Cell incubation time 18-48 hours			**B2**

A. Plate preparation (sterile conditions)

1. Remove the plate from the sealed package and wash the plate three times with sterile PBS (200 µl/well).
2. Block/condition the plate by adding cell incubation medium containing 10% fetal calf serum (200 µl/well). Incubate for at least 30 minutes at room temperature.

B. Cell incubation (sterile conditions)

1. Remove the medium and add the stimuli followed by the cell suspension. Cells and stimuli can also be mixed before addition to the plate.
2. Incubate the cells in the plate at 37°C in a humidified incubator with 5% CO_2. Approximate incubation time is indicated above and can be further optimized by the user. Do not move the plate during incubation and avoid evaporation, for example by wrapping the plate in aluminum foil.

C. Detection

1. Remove the cells by emptying the plate and then wash the plate five times with PBS (200µl/well).
2. Dilute the detection antibodies in PBS containing 0.1% BSA (PBS-0.1% BSA) according to the table above. If more than one analyte is analyzed, dilute the detection antibodies in the same tube. Add 100 µl/well and incubate for 2 hours at room temperature.
3. Wash the plate five times with PBS (200 µl/well).
4. Dilute the fluorophore-conjugates in PBS-0.1% BSA to the concentrations as shown in the table above. If more than one analyte is analyzed, dilute the conjugates in the same tube. Add 100 µl/well and incubate for 1 hour at room temperature. Protect the plate from light throughout the assay.
5. Wash the plate five times with PBS (200 µl/well).
6. Empty the plate, add Fluorescence enhancer (50 µl/well), and leave for 5-15 minutes at room temperature.
7. Empty by flicking the plate to remove the Fluorescence enhancer. Do not wash. It is important that the plate is not tapped against paper towels since dust particles may interfere with the assay.
8. Remove the underdrain (the soft plastic drain under the plate), for example using pliers. Dry the plate protected from light. The plate should be completely dry before analysis. Store plate in the dark at room temperature.
9. Spot analysis is performed with an automated FluoroSpot reader equipped with filters for the fluorophores used. Filters should have high specificity to avoid bleed-through artifacts.

Analyte	Required filter
IL-22	Excitation 380 nm/emission 430 nm (LED380)
IFN-γ	Excitation 490 nm/emission 510 nm (LED490)
IL-10	Excitation 550 nm/emission 570 nm (LED550)
IL-17A	Excitation 640 nm/emission 660 nm (LED640)

TIPS FOR A SUCCESSFUL ASSAY

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

Washing
Plates can be washed using a multi-channel micropipette. A regular plate washer can be used in washing steps when sterile conditions are not required, provided that the washing head is adapted to ELISpot/FluoroSpot plates.

Serum
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.

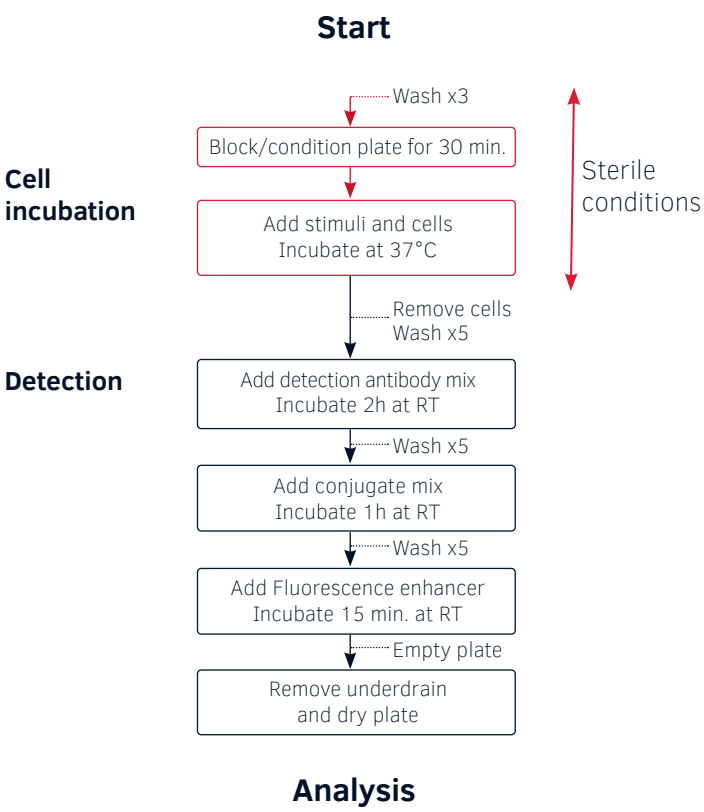
Cells
Both freshly prepared and cryopreserved cells may be used in FluoroSpot. We recommend that cryopreserved cells are rested for at least 1 hour after thawing to allow removal of cell debris before addition to the plate. Triplicates or duplicates of 200,000-500,000 cells/well are usually recommended to assess antigen-specific responses. For polyclonal activators, the cell number should be reduced in order to avoid confluent spot formation.

Assay controls
The number of cells responding to antigen 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. A polyclonal activator such as anti-CD3 mAb (included in the kit) or phytohemagglutinin (1-10 µg/ml) is often included as a control for cell viability and functionality of the assay.

Co-stimulation with anti-CD28
Anti-CD28 mAb provides a co-stimulatory signal to antigen-specific responses by binding to CD28 on T cells. Addition of an anti-CD28 mAb together with antigen (step B1) can be used to enhance antigen-specific responses. However, if the concentration of anti-CD28 mAb is too high, non-specific cytokine secretion may be elevated. Anti-CD28 can be used to circumvent capture effects, which can occur when different capture antibodies are coated in the same well, where absorption of one cytokine may negatively affect the secretion of another cytokine. See www.mabtech.com for more information about capture effects.

Analysis
An automated FluoroSpot reader with separate filters is required for analysis. The reader should be equipped with filters for the fluorophores used. Filters should be selective for the specific wavelengths to avoid bleed-through artifacts. Spots from cells secreting multiple analytes are identified by coordinates, i.e. based on spot position. In order to obtain accurate measurements of cells secreting multiple analytes, the automated reader must be able to create an overlay analysis from the filters. To avoid fading, the plates should be kept dark and should preferably be analyzed within 1 week of development.

PROTOCOL SUMMARY



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