FluoroSpot^{BASIC} Protocol

General guideline for analysis of protein-secreting cells with FluoroSpot. Please consult the analyte-specific FluoroSpot^{BASIC} data-sheets for recommended concentrations and cell incubation times.

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Protocol Introduction

This FluoroSpot protocol can be used to detect cells secreting one or multiple analytes. For multi-parameter FluoroSpot, cell numbers and kinetics for all analytes need to be considered.

A. Coating (sterile conditions)

1. In the same tube, dilute the capture antibody/antibodies in sterile PBS to the concentrations provided in the analyte-specific datasheet. For optimal results, filter all antibody/conjugate dilutions throughout the assay using a 0.2 µm low protein binding filter.
2. Pre-wet the membrane of an IPFL plate with 35% EtOH, 15 µl/well, for maximum 1 minute. It is essential that the membrane is not allowed to dry after the treatment.
3. Wash the plate 3 times with sterile water, 200 µl/well.
4. Add the capture antibody solution, 100 µl/well, and incubate overnight at 4-8°C.

B. Cell Incubation (sterile conditions)

1. Wash the plate 3 times with sterile PBS, 200 ul/well.
2. Block/condition the plate by adding 200 µl/well of cell incubation medium. Medium supplemented with e.g 10% fetal calf serum or serum-free medium containing BSA or HSA as protein source may be used. Incubate for at least 30 minutes at room temperature.
3. Remove the medium and add the stimuli followed by the cell suspension. Cells and stimuli can also be mixed before addition to the plate. Both freshly prepared and cryopreserved cells may be used. It is recommended that the latter are rested for at least one hour to allow removal of cell debris.

   For enumeration of antigen-specific T-cell responses, cell numbers typically range from 200,000-400,000 cells/well. Lower cell numbers are recommended for polyclonal activators such as PHA, ConA or anti-CD3 mAb which are often used as positive controls for T cells. To compensate for possible capture effects and to increase secretion of certain cytokines, a co-stimulatory anti-CD28 antibody (product code 3608-1-50) can be used.
4. Incubate the cells in the plate at 37°C in a humidified incubator with 5% CO_2. Approximate incubation times are indicated in the datasheet and can be further optimized by the user. Do not move the plate during incubation and avoid evaporation e.g. by wrapping the plate in aluminium foil.

C. Detection

1. Remove the cells by emptying and washing the plate 5 times with PBS, 200 µl/well. An ELISA plate washer can be used for these non-sterile washing steps, provided that the washer head has been adapted to FluoroSpot and ELISpot plates. Do not include Tween or other detergents in the buffers.

2. In the same tube, dilute the detection antibody/antibodies in PBS containing 0.1% BSA (PBS-0.1% BSA) to the concentrations provided in the analyte-specific datasheet. Filter the mixture using a 0.2 µm low protein binding filter. Add 100 µl/well and incubate for 2 hours at room temperature.

3. Wash the plate 5 times with PBS, 200 µl/well.

4. In the same tube, dilute the fluorophore-conjugated reagents in PBS-0.1% BSA to the concentrations provided in the analyte-specific datasheet. Filter the mixture using a 0.2 µm low protein binding filter. Add 100 µl/well and incubate for 1 hour at room temperature.

5. Wash the plate 5 times with PBS, 200 µl/well.

6. Empty the plate and add Fluorescence enhancer, 50 µl/well, and leave the plate for 15 minutes at room temperature.

7. Empty the plate and remove any residual Fluorescence enhancer by firmly tapping the plate against clean paper towels.

8. Remove the underdrain (the soft plastic under the plate). 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. Spots from double and triple secreting cells are identified by coordinates i.e., based on spot position. Fluorescent spots may fade due to excessive exposure to light and it is recommended that the plate is analyzed within one week of development.

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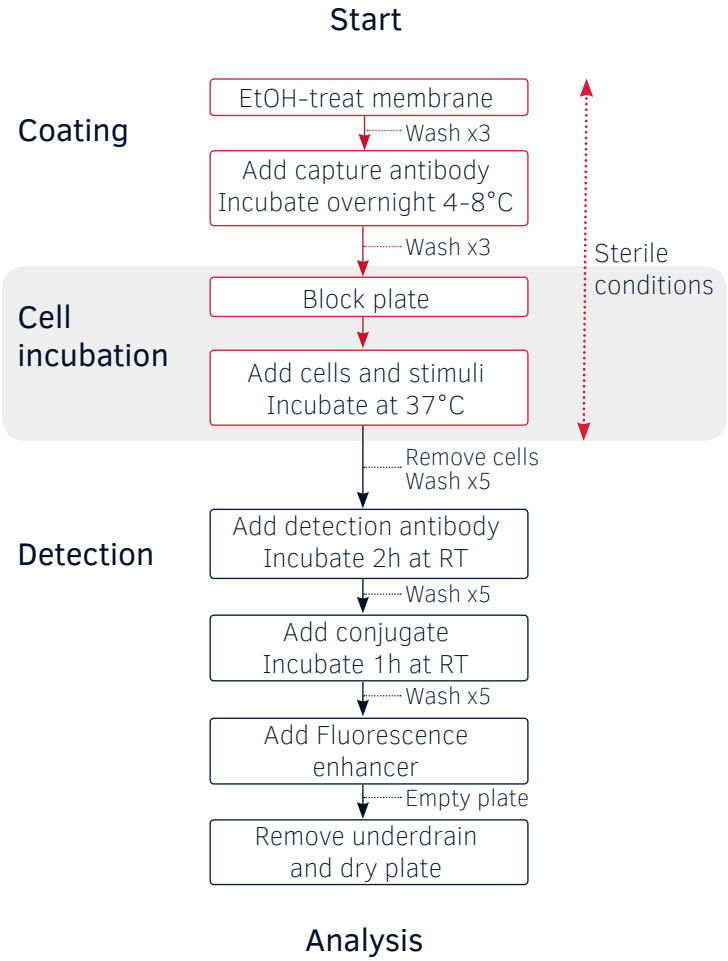
FluoroSpot Overview



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