Mouse IFN gamma ELISPOT Kit

Introduction

IFN-γ (interferon-γ) is a type II interferon family cytokine involved in antiviral, antibacterial, and antitumor responses. In addition, IFN-γ acts as an anti-inflammatory mediator by promoting the development of regulatory T cells and inhibiting Th17 cell differentiation. IFN-γ dimers signal through a receptor complex of two IFN-γ R1 and two IFN-γ R2 subunits.

Enzyme-linked immunospot (ELISPOT) technology is the best technology in the world to detect the level of cellular immunity in organisms. It combines high sensitivity, high confidence, high throughput, single-cell level, functional assays, and low cost. This ELISpot assay uses a capture antibody specific for mouse IFN-γ that is pre-coated onto a PVDF-supported microplate. Appropriately stimulated cells are pipetted directly into the wells, and immobilized antibodies near secreting cells are bound to secreted mouse IFN-γ. After the washing step and incubation with the biotinylated detection antibody, horseradish oxidase conjugated to streptavidin is added. The unbound enzymes are subsequently removed by washing and a substrate solution (AEC solution) is added. A red precipitate is formed at the cytokine localization site and appears as spots, each individual spot representing a single mouse IFN-γ secretory cell, and finally counted using an automated ELISpot reader system.

Components and Storage

Components \ Size	96 Tests	Storage
Pre-coated plate (96-well, undetachable)	5 plates	4°C
250× Positive Stimulus	20 μL	4°C
20× Washing Solution	250 mL	4°C
Biotin-Anti-IFNγ Antibody	25 μL	4°C
Streptavidin-HRP	10 μL	4°C
Antibody Dilution Buffer	120 mL	4°C
AEC Solution	0.6 mL	4°C away from light
Acetate Buffer	0.6 mL	4°C
Shipping: Blue ice	Shelf life: 12 months	

Protocol

Day 1 Operations:

1. Pre-coated plate (96-well, undetachable) activation: Add 200 μL of serum-free medium to each well and let it stand for 5-10 minutes at room temperature before pulling it out.
2. Add the cell suspension: Add the adjusted concentration of the cell suspension to each experimental well, 100 μL/well. Positive control wells: 1×10^5 cells/well can be used for cell concentration; Negative control wells: 1×10^5 cells/well can be used for cell concentration; Background negative control: medium for resuspending cells (serum-free medium) was added; Experimental wells: The cell concentration of the sample is adjusted by the experimenter according to the experiment.
3. Add stimulant: 10 μL/well as follows: Positive control well: Add positive stimulant (serum-free medium formulated to a final concentration of 10×). Negative control wells (with background negative control wells): Add serum-free medium (or medium for resuspending cells). Experimental wells: Add the experimenter's own stimulus (serum-free medium formulated to a 10× final concentration).
4. Incubation: After all samples and stimuli have been added, cover the plate. Place in a 37°C, 5% CO_2 incubator for 16-20 h.

Next day procedure (aseptic procedure is no longer required):

5. Lyse cells: Pour the cells and medium in the wells. Add cold ddH_2O, 200 μL/well, and place in a 4°C freezer for 10 minutes to lyse the cells with hypotonic lysis.
6. Wash the plate: Pour the liquid in the wells, 20×Washing Solution, dilute with ddH_2O to 1×, 200 μL/well, wash 5 times. Stay for 30-60 s each. For the last time, buckle dry on absorbent paper.
7. Biotin antibody incubation: Add Biotin-Anti-IFNγ Antibody (1:2000 dilution with Antibody Dilution Buffer), 100 μL/well, and incubate for 1 h at room temperature.
8. Wash the plate: Step 6 is the same as in the experiment.
9. Streptomycin HRP incubation: Streptavidin-HRP (diluted 1:5000 with diluent), 100 μL/well, incubated for 1 h at room temperature.
10. Plate washing: After incubation, wash with PBS, 200 μL/well, 5 washes. Stay for 30-60 s each. For the last time, buckle dry on absorbent paper.
11. Chromogenic development: Add AEC Solution and Acetate Buffer to ddH_2O, mix well at a dilution ratio of 1:100, 100 μL/well, and protect from light for 10-30 min. If there is no background, color development can continue. until the color development is satisfactory.
12. Terminate color development: pour the liquid in the hole, uncover the base of the plate, wash the front and back sides and the base 3-5 times with ddH_2O/tap water to stop color development. Place the plate in a cool place at room temperature and allow it to dry naturally before closing the base.
13. ELISPOT plate spot counting and record various parameters of the spot for statistical analysis.

Note

1. The operation is strictly in accordance with the instructions, and the kits with different batch numbers of this product cannot be mixed.
2. Store all reagents in each group at 4°C and equilibrate to room temperature before use.
3. It is normal for crystallization to precipitate after 20× concentrated washing solution is stored at 4°C, and it can be placed at room temperature and gently shaken and mixed before use, which has no effect on the experimental results.
4. The chromogenic solution of this substrate should be stored in the dark and should not be exposed to strong light.
5. This product is for scientific research use only.

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