Human IFN gamma ELISPOT

Catalog Number 88-7386

Pub. No. MAN0017483 **Rev.** A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product information

Symbol	Contents	Human IFN gamma ELISPOT
REF	Catalog number	88-7386
	Temperature limitation	Store at 2–8°C
LOT	Batch code	Refer to vial
	Use by	Refer to vial
⚠	Caution	Contains sodium azide

Description

This Human IFN gamma ELISPOT reagent set contains the necessary reagents for performing enzyme-linked immunosorbent spot (ELISPOT) assays for high-resolution frequency analysis of IFN gamma-secreting cells. This ELISPOT reagent set is pre-titrated for optimal spot development.

Time requirements

- 1 overnight incubation
- 1–2 days cell activation
- 3–5 hours washing, antibody incubations, and color development

Components

- **Capture Antibody:** Pre-titrated, functional grade (low endotoxin) purified antibody
- **Detection Antibody:** Pre-titrated, biotin-conjugated antibody
- **ELISA/ELISPOT Coating Buffer**

  ELISA/ELISPOT Coating Buffer Powder or 10X PBS ELISPOT Coating Buffer
- **5X ELISA/ELISASPOT Diluent**
- **Enzyme**

  Pre-titrated Avidin-HRP Concentrate
- **Certificate of Analysis:** Lot-specific instructions for dilution of antibodies and enzyme

Other materials needed

- **Reagents:**
  - 96-Well PVDF Membrane ELISPOT Plates (Milipore, Cat. No. MAIPS4510)
  - AEC (3-amino-9-ethyl carbazole) Substrate (Sigma, Cat. No. A-5754
  - Distilled water (dH_2O)
  - ELISPOT Wash Buffer: ELISA/ELISPOT Wash Buffer Powder (Cat. No. 00-0400) or 1X PBS with 0.05% Tween™ 20
  - Complete RPMI-1640
  - 1X PBS
- **Instruments:**
  - Pipettes and pipettors
  - Refrigerator
  - Incubator
  - Laminar flow hood
  - Plate washer: Wash bottle or automated wash machine
  - ELISPOT plate reader or dissecting microscope for visual inspection

Reagent preparation

- **ELISA/ELISPOT Coating Buffer (1X)**

  Make a 1:10 dilution of 10X PBS ELISPOT Coating Buffer in dH_2O or reconstitute ELISA/ELISPOT Coating Buffer Powder to 1L in dH_2O and sterilize by filtering using a 0.22-µM filter.
- **Complete RPMI-1640**

  Complete RPMI-1640 with 10% Fetal Bovine Serum and 1% Penicillin/Streptomycin/L-Glutamine.
- **5X ELISA/ELISPOT Diluent**

  Dilute 5X ELISA/ELISPOT Diluent 1:5 in dH_2O.
- **ELISA/ELISPOT Wash Buffer**

  Reconstituted ELISA/ELISPOT Wash Buffer Powder (Cat. No. 00-0400) or 1X PBS with 0.05% Tween™ 20 (0.5 mL Tween™ 20 in 1 L PBS).
- **0.1M Acetate Solution (pH 5.0)**
  a. Combine 148 mL of 0.2 M acetic acid (11.55 mL glacial acetic acid per 1 L of dH_2O) with 352 mL of 0.2 M sodium acetate (27.2 g sodium acetate per 1 L of dH_2O).
  b. QS to 1 L with dH_2O, then adjust pH to 5.0.
- **AEC (3-amino-9-ethyl carbazole) Substrate Solution**
  a. Prepare AEC Stock Solution by dissolving 100 mg of AEC in 10 mL of N, N Dimethylformamide (DMF; Cat. No. 20672).
  b. Add 333 µL of AEC Stock Solution to 10 mL of 0.1M Acetate Solution (pH 5.0), then filter through a 0.45-µm filter.
  c. Add 5 µL of 30% H_2O_2 just before use. Mix and use immediately.

Experimental procedures

Aseptic steps

Note: Use sterile buffers and aseptic techniques and perform all steps in a laminar flow hood.

1. Dilute Functional Grade purified capture antibody in sterile ELISA/ELISPOT Coating Buffer, as noted on the Certificate of Analysis.
2. Coat ELISPOT plate with 100 µL/well of capture antibody solution, then incubate at 2-8°C overnight.
3. Decant or aspirate coating antibody from plate.
4. Wash plates 2 times with 200 µL/well of sterile ELISA/ELISPOT Coating Buffer, then decant.
5. Block plate with 200 µL/well of complete RPMI-1640 at room temperature for 1 hour, then decant or aspirate plate.
6. Aliquot mitogen, antigen, or controls diluted in complete RPMI-1640 to appropriate wells at 100 µL/well.

7. Aliquot cells at desired densities (e.g., $1x10^5$–$2x10^6$ cells/mL) at 100 µL/well and incubate at 37°C, in a 5% CO2 humidified incubator for 24-48 hours.

   Note: Optimal kinetics and cell densities vary with target cytokine, treatment, and cell type and must be empirically determined. Cells can be diluted in a sterile tissue culture plate starting at $2x10^6$ cells/well in triplicate wells with a series of 1:3 or 1:4 serial dilutions down the plate, and then transferred to the ELISPOT plate.

Non-aseptic steps

1. Decant cells and medium from plates.
2. Wash plate 3 times with ELISA/ELISPOT Wash Buffer.
3. Dilute biotinylated detection antibody in Assay Diluent according to instructions on the Certificate of Analysis.
4. Add 100 µL/well of detection antibody solution, then incubate at room temperature for 2 hr (or at 2-8°C overnight).
5. Decant antibody solution, then wash 4 times with ELISA/ELISPOT Wash Buffer allowing wells to soak for 1 minute for each wash.
6. Dilute Avidin-HRP reagent in Assay Diluent according to instructions on the Certificate of Analysis.
7. Add 100 µL/well of Avidin-HRP solution, then incubate at room temperature for 45 minutes.
8. Decant Avidin-HRP solution.
9. Wash plate 3 times with ELISA/ELISPOT Wash Buffer.
10. Wash 2 times with 1X PBS without Tween™ 20.
11. Add 100 µL/well of freshly-prepared AEC Substrate Solution, then develop at room temperature for 10-60 minutes monitoring development of spots.
12. Stop the substrate reaction by washing wells 3 times with 200 µL/well of dH_2O.
13. Air-dry the plate, then count spots using a dissecting microscope or automated ELISPOT plate reader.

    Store plates in the dark prior to reading.

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- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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