Mouse IL-4 ELISPOT

Catalog Number: 88-7844
Also known as: Interleukin-4
RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

REF **Contents:** Mouse IL-4 ELISPOT
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Temperature Limitation: Store at 2-8°C.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial
Contains sodium azide

Description

This Mouse IL-4 ELISPOT Ready-SET-Go! reagent set contains the necessary reagents for performing enzyme linked immunosorbent spot (ELISPOT) assays for high resolution frequency analysis of IL-4-secreting cells. This ELISPOT reagent set is pre-titrated for optimal spot development.

Components

Capture Antibody. Pre-titrated, Functional Grade (low endotoxin) purified antibody
Detection Antibody. Pre-titrated, biotin-conjugated antibody
ELISA/ELISPOT Coating Buffer Powder. This Ready-Set-Go! ELISPOT Set may contain ELISA/ELISPOT Coating Buffer Powder (Reconstitute to 1L with dH20 and filter (0.22 uM)) or 10X PBS ELISPOT Coating Buffer (Dilute 1 part 10X Buffer into 9 parts dH20 and filter with 0.22 uM).
Assay Diluent. 5X concentrated
Detection enzyme. Pre-titrated Avidin-HRP
Certificate of Analysis. Lot-specific instructions for dilution of antibodies and enzyme

References

Anis MM, Fulton SA, et al. 2007. Modulation of naive CD4+ T-cell responses to an airway antigen during pulmonary mycobacterial infection. Infect Immun. 75(5):2260-8. (ELISPOT kit, PubMed)

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invitrogen

ELISPOT Protocol

Protocol: ELISPOT

Materials Provided

- Please refer to the Certificate of Analysis (C of A) for components

Other Materials Needed (see note at the end of the protocol)

- 96-Well PVDF Membrane ELISPOT Plates (Millipore, Cat. No. MAIPS4510)
- AEC (3-amino-9-ethyl carbazole) Substrate (Sigma, Cat. No. A-5754)
- Distilled water (dH_2O)
- ELISPOT Wash Buffer: 1X PBS, with 0.05% Tween-20 (or Thermo Fisher ELISA/ELISPOT Wash Buffer Powder, Cat. No. 00-0400)
- 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

Time Requirements

- 1 overnight incubation
- 1-2 day cell activation
- 3-5 hour washing, antibody incubations and color development

Experimental Procedure

Aseptic Steps:

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

1. Dilute Functional Grade purified capture antibody in sterile ELISA/ELISPOT Coating Buffer, as noted on Certificate of Analysis which is included with the reagent set. Coat ELISPOT plate with 100 µL/well of capture antibody solution. Incubate at 2-8°C overnight.
2. Decant or aspirate coating antibody from plate.
3. Wash plates 2 times with 200 µL/well of sterile ELISA/ELISPOT Coating Buffer. Decant.
4. Block plate with 200 µL/well of complete RPMI-1640 at room temperature for 1 hour. Decant or aspirate plate.
5. Aliquot mitogen, antigen, or controls diluted in complete RPMI-1640 to appropriate wells at 100 µL/well. Aliquot cells at desired densities (e.g., $1x10^5$/mL - $2x10^6$/mL) at 100 µL/well and incubate at 37°C, in a 5% CO_2 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. See references. Cells can be diluted in a sterile tissue culture plate starting at $2x10^6$/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:

6. Decant cells and medium from plates. Wash plate 3 times with ELISA/ELISPOT Wash Buffer.
7. Dilute biotinylated detection antibody in Assay Diluent according to instructions on the Certificate of Analysis provided with the reagent set. Add 100 µL/well of detection antibody solution. Incubate at room temperature for 2 hr (or at 2-8°C overnight).

8. Decant antibody solution. Wash 4 times with ELISA/ELISPOT Wash Buffer. Allow wells to soak for 1 minute for each wash.
9. Dilute Avidin-HRP reagent in Assay Diluent according to instructions on the Certificate of Analysis provided with the reagent set. Add 100 µL/well of Avidin-HRP solution and incubate at room temperature for 45 minutes.
10. Decant Avidin-HRP solution. Wash plate 3 times with ELISA/ELISPOT Wash Buffer, and then 2 times with 1X PBS (no Tween-20).
11. Add 100 µL/well of freshly-prepared AEC Substrate Solution and develop at room temperature for 10-60 minutes; monitor development of spots.
12. Stop the substrate reaction by washing wells 3 times with 200 µL/well of distilled water.
13. Air-dry the plate. Count spots using a dissecting microscope or automated ELISPOT plate reader. Store plates in the dark prior to reading.

Solutions

ELISA/ELISPOT Coating Buffer Powder:

- Reconstitute powder to 1 L in dH_20; filter sterilize using a 0.22 µM filter

Complete RPMI-1640:

- RPMI-1640 with 10% Fetal Bovine Serum and 1% Pennicillin/Streptomycin/L-Glutamine

Assay Diluent (supplied as 5X)

- Dilute 5X solution to 1X in dH_2O

ELISA/ELISPOT Wash Buffer:

- 1X PBS with 0.05% Tween-20 (0.5 mL Tween-20 in 1 L PBS) or Thermo Fisher ELISA Wash Buffer Powder (Cat. No. 00-0400)

AEC (3-amino-9-ethyl carbazole) Substrate Solution:

- AEC Stock Solution: Dissolve 100 mg of AEC in 10 mL of N,N Dimethylformamide (DMF; Pierce, Cat. No. 20672)
- Add 333 µL of AEC Stock Solution to 10 mL of 0.1 M Acetate Solution (pH 5.0) (see below for recipe). Filter through a 0.45 µm filter.
- Just before use, add 5 µL of 30% H_2O_2. Mix and use immediately.

0.1 M Acetate Solution (pH 5.0):

- 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).
- QS to 1 L with dH_2O. Adjust pH to 5.0.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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