Mouse IL-21 ELISPOT

Catalog Number: 88-7210
Also known as: Interleukin-21
RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: Mouse IL-21 ELISPOT
REF **Catalog Number:** 88-7210

Temperature Limitation: Store at 2-8°C.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial

Description

IL-21 is a 17 kDa immunomodulatory cytokine produced mainly by NKT, T helper (Th) 17 and T follicular helper (TFH) cells. In TFH cells, IL-21 expression leads to autocrine signaling through the IL-21 receptor (IL-21R) and STAT3, which leads to additional transcriptional activation by Bcl-6. As with IFN gamma for Th1, IL-4 for Th2 cells, and IL-17A for Th17 cells, IL-21 is critical for TFH effector function. This cytokine plays a role in T cell-dependent B cell differentiation into plasma cells and memory cells, stimulation of IgG production and induction of apoptotic signaling in naïve B cells.

In Th17 cells, IL-21 expression and autocrine feedback through STAT3, IRF4 and ROR gamma t lead to upregulation of the IL-23R, thereby preparing Th17 cells for maturation and maintenance by the inflammatory cytokine IL-23. While upregulating IRF4 and ROR gamma t, IL-21 also mediates the downregulation of Foxp3. High levels of IL-21 are present in chemically-induced colitis models. IL-21-deficient mice are protected from developing colitis upon chemical treatment by their inability to upregulate Th17-associated molecules.

Components

Capture Antibody. Pre-titrated, Functional Grade (low endotoxin) purified antibody
Detection Antibody. Pre-titrated, biotin-conjugated antibody
ELISA/ELISPOT Coating Buffer. 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 the dilution of antibodies and enzyme

Applications Reported

This ELISPOT set is for the high resolution frequency analysis of IL-21 secreting cells.

Applications Tested

This mouse IL-21 ELISPOT Ready-SET-Go! set contains all of the necessary reagents for performing enzyme-linked immunosorbent spot (ELISPOT) assays for high resolution frequency analysis of IL-21 secreting cells. The reagents in this set have been pre-titrated for optimal spot development. Millipore Multiscreen HTS 96-well filtration plates are recommended, but not included, for use in this assay.

This assay has been validated for the detection of endogenous IL-21 using Th17-polarized Balb/c splenocytes. Splenocytes were cultured in the presence of Anti-Mouse CD3e Functional Grade Purified (cat. 16-0031), Anti-Mouse CD28 Functional Grade Purified (cat. 16-0281), Anti-Mouse IL-2 Functional Grade Purified (cat. 16-7022), Anti-Mouse IL-4 Functional Grade Purified (cat. 16-7041), Anti-Mouse IFN gamma Functional Grade Purified (cat. 16-7311), Human TGF beta 1 Recombinant Protein (cat. 14-8348), and Mouse IL-6 Recombinant Protein (cat. 14-8061) for three days to induce Th17 polarization. The cells were then stimulated with PMA and Ionomycin and transferred to coated plates for the analysis of IL-21 secretion.

References

Konforte D, Paige CJ. Interleukin-21 regulates expression of the immediate-early lytic cycle genes and proteins in Epstein-Barr Virus infected B cells. Virus Res. 2009 Sep;144(1-2):339-43.

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Pot C, Jin H, Awasthi A, Liu SM, Lai CY, Madan R, Sharpe AH, Karp CL, Miaw SC, Ho IC, Kuchroo VK. Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. J Immunol. 2009 Jul 15;183(2):797-801.

Elsaesser H, Sauer K, Brooks DG. IL-21 is required to control chronic viral infection. Science. 2009 Jun 19;324(5934):1569-72.

Bauquet AT, Jin H, Paterson AM, Mitsdoerffer M, Ho IC, Sharpe AH, Kuchroo VK. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. Nat Immunol. 2009 Feb;10(2):167-75.

Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS, Jetten AM, Dong C. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature. 2007 Jul 26;448(7152):480-3.

Related Products
12-7211 eBioscience™ Anti-Mouse IL-21 PE (FFA21)
16-7211 eBioscience™ Anti-Mouse IL-21 Functional Grade Purified (FFA21)
88-8210 Mouse IL-21 Uncoated ELISA Kit

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

Limited product warranty

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