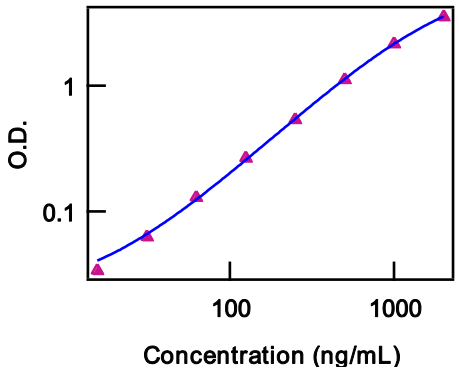
Mouse IL-17E (IL-25) ELISA Ready-SET-Go!®

Catalog Number: 88-7002
Also known as: Interleukin-17E, Interleukin-25
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Mouse IL-17E (IL-25) ELISA Ready-SET-Go!®

Product Information

Contents: Mouse IL-17E (IL-25) ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7002
Sensitivity: 16 pg/mL
Standard Curve Range: 16 - 2000 pg/mL

Temperature Limitation: Store at 2-8°C except standard which should be stored at less than or equal to -70°C.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial

Description

This Mouse IL-17E ELISA Ready-SET-Go! reagent set contains all of the necessary buffers and reagents to perform enzyme-linked immunosorbent assays. The set has been optimized for the accurate and precise measurement of mouse IL-17E in serum and tissue culture supernatant samples. Serum samples must be diluted 8-fold in the provided sample diluent prior to evaluation in this assay.

IL-17E, or IL-25, was first identified as an IL-17 family member on the basis of sequence homology. It was originally believed to be a Th2 cytokine based on the detection of mRNA transcripts in polarized Th2 cells, but expression was later observed in epithelial cells, eosinophils, and mast cells. Later studies confirmed the role of IL-17E as a potent inducer of type 2 immune responses. IL-17E signaling induces the differentiation of CD4+ T cells towards the Th2 lineage and upregulates expression of Th2 cytokines such as IL-4, IL-5, and IL-13. Administration of IL-17E also promotes symptoms of allergic asthma, such as eosinophil infiltration, airway hyperreactivity, and high circulating levels of IgE. IL-17E binds to IL-17BR, a receptor shared with IL-17B, and activates NF kappa-B pathways.

Components

Capture Antibody. Pre-titrated, purified antibody
Detection Antibody. Pre-titrated, biotin-conjugated antibody
Standard. Recombinant cytokine for generating standard curve and calibrating samples
Coating Buffer. 10X PBS ELISA Coating Buffer
Assay Diluent. 5X Concentrated
Sample Diluent B. 12 mL of a 1X solution per plate
Detection Enzyme. Pre-titrated Avidin-HRP
Substrate Solution. Tetramethylbenzidine (TMB) Solution
Certificate of Analysis. Lot-specific instructions for dilution of antibodies and standards.
96-well Plates. Corning Costar flat-bottom plates (included with Cat. #s ending in -22 and -86)

Applications Reported

This ELISA set is for the quantitative detection of mouse IL-17E in serum, plasma, and tissue culture supernatant

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samples.

Applications Tested
This assay has been validated for the detection of endogenous mouse IL-17E with supernatant collected from PMA and Ionomycin-stimulated culture of Balb/c bone marrow-derived mast cells. No detectable levels were observed in unstimulated cultures.

This assay was evaluated for specificity on a panel of 72 recombinant cytokines at 100 ng/mL. No significant cross-reactivity was observed, including to Human IL-17E Recombinant Protein, or to other recombinant proteins of the IL-17 family.

It is recommended that serum samples be diluted at least 8-fold with the included Sample Diluent B in order to ensure optimal recovery. Sample Diluent B is also compatible with tissue culture supernatant samples, although it is not necessary to use this buffer for their evaluation. Plasma samples have not been tested in this assay.

References
Dong C. Regulation and pro-inflammatory function of interleukin-17 family cytokines. Immunol Rev. 2008 Dec;226:80-6.

Kang CM, Jang AS, Ahn MH, Shin JA, Kim JH, Choi YS, Rhim TY, Park CS. Interleukin-25 and interleukin-13 production by alveolar macrophages in response to particles. Am J Respir Cell Mol Biol. 2005 Sep;33(3):290-6.

Ikeda K, Nakajima H, Suzuki K, Kagami S, Hirose K, Suto A, Saito Y, Iwamoto I. Mast cells produce interleukin-25 upon Fc epsilon RI-mediated activation. Blood. 2003 May 1;101(9):3594-6.

Hurst SD, Muchamuel T, Gorman DM, Gilbert JM, Clifford T, Kwan S, Menon S, Seymour B, Jackson C, Kung TT, Brieland JK, Zurawski SM, Chapman RW, Zurawski G, Coffman RL. New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. J Immunol. 2002 Jul 1;169(1):443-53.

Related Products
14-8175 Mouse IL-17E (IL-25) Recombinant Protein

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TDS Protocol

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Other Materials Needed

- Buffers
  - Wash Buffer: 1 x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, cat 00-0400)
  - Stop Solution: 1M H_3PO_4 or 2N H_2SO_4
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning Costar 9018)

  NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp 96 well plates provided or suggested.
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

NOTE: To ensure optimal results from this ELISA Ready-SET-Go! set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.

Stability

This ELISA set is guaranteed to perform as specified at least 12 months from date of receipt if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents.

Storage Instructions for Cytokine Standards

The frozen cytokine standard is already aliquoted at 20 µl per vial. Upon receipt, frozen cytokine standard should be immediately stored at -80°C; stable for at least 12 months. After thawing, quick-spin vial prior to opening. Do not re-aliquot into smaller fractions. These are single use vials. Use one time and discard. For dilution of the standard, please see instructions on the Certificate of Analysis and follow these as written.

Storage Instructions for Other Set Reagents

Store at 4°C.

Time Requirements

- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

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TDS Protocol

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Experimental Procedure

1. Coat Corning Costar 9018 ELISA plate with 100 µl/well of capture antibody in Coating Buffer (dilute as noted on Certificate of Analysis, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 5 times with >250 µl/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µl/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
5. Using Sample Diluent B, dilute standards as noted on the Certificate of Analysis (C of A). Add 100 µl/well of standard to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve. Add 100 µl/well of your samples to the appropriate wells. Serum and plasma samples must be diluted at least 8-fold in Sample Diluent B prior to evaluation in this assay. Cover or seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).
6. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
7. Add 100 µl/well of detection antibody diluted in 1X Assay Diluent* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 5 washes.
9. Add 100 µl/well of Avidin-HRP* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 7 washes.
11. Add 100 µl/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µl of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

***NOTE: Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.**

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TDS Protocol

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Standard Calibration

The standard of the Ready-SET-Go! is calibrated against NIBSC standards:

Table of Standard Calibration				
Cytokine	ng of eB standard	ng of NIBSC standard	U of NIBSC standard	NIBSC Lot #
hIL-2	1	1.1	14.6	86/564
hIL-4	1	2.2	22	88/656
hIL-5	1	2.2	22	90/586
hIL-6	1	1.7	170	89/548
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIFN-g	1	1.1	22	87/586
hTNF-a	1	0.9	36	87/650
mIL-2	1	3.1	310	93/566
mIL-4	1	3	30	91/656
mIL-6	1	8.5	850	93/730
mIFN-g*	1		4.5	Gg02-901-533
mTNF-a	1	1.7	340	88/532
* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)				

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
A. High Background	1. Improper and inefficient washing	1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed
	2. Cross contamination from other specimens or positive control	2. Repeat ELISA, be careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat test using correct dilutions; check with the recommendations of the antibody manufacturer
B. No signal	1. Improper, low protein binding capacity plates were used	1. Repeat ELISA, using recommended high binding capacity plates
	2. Wrong substrate was used	2. Repeat ELISA, use the correct substrate
	3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity	3. Repeat ELISA, make sure your system contains no enzyme inhibitor

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C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly
	2. Incorrect dilutions of standard	2. Follow recommendations of standard handling exactly as written on the certificate of analysis
	3. Insufficient incubation time	3. Repeat ELISA, follow the protocol carefully for each step□s incubation time
	4. Incorrect storage of reagents	4. Store reagents in the correct temperature, avoid freeze and thaw, avoid using the □frost free□ freezer
	5. Wrong filter in ELISA reader was used	5. Use the correct wavelength setting
	6. Wrong plate used	6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates
D. Variation amongst replicates	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly; see certificate of analysis
	2. Poor mixing of samples	2. Mix samples and reagents gently and equilibrate to proper temperature
	3. Plates not clean	3. Plates should be wiped on bottom before measuring absorbance
	4. Improper, low binding capacity plates were used	4. Use recommended high binding capacity plates
	5. Reagents have expired	5. Do not use if past expiration date

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