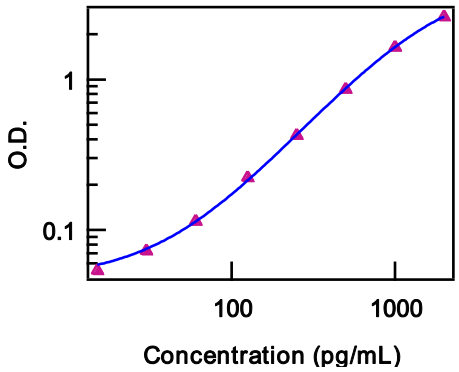
Mouse IL-27 ELISA Ready-SET-Go!®

Catalog Number: 88-7274
Also known as: Interleukin-27
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Mouse IL-27 ELISA Ready-SET-Go!®

Product Information

Contents: Mouse IL-27 ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7274
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-27 Ready-SET-Go! ELISA Set (with or without high affinity binding ELISA microwell plates) contains the necessary reagents, buffers and diluents for performing quantitative enzyme linked immunosorbent assays (ELISA). This ELISA reagent set is specifically engineered for accurate and precise measurement of mouse IL-27 protein levels from samples including serum, and supernatants from cell cultures.This ELISA has 11.8% cross reactivity to the recombinant human IL-27 and no cross reactivity to the recombinant mouse IL-12 when spiked in excess (100ng/ml).

IL-27, a member of the IL-12 family, is a heterodimeric protein consisting of the p40-related protein Epstein-Barr virus-induced gene 3 (EBI3) non-covalently linked to an IL-12p35-related protein, p28 (also known as IL-30). IL-27 is produced by activated antigen presenting cells and mature dendritic cells. LPS-stimulated IL-27 production is dependent on the TLR4/MyD88 mediated pathway. IL-27 prevents the development of Th17 cells even in the presence of TGF-beta and IL-6, but it has little or no effect on committed Th17 cells. Recent studies indicate that IL-27 has potent anti-tumor activity. In vitro, IL-27 has been found to act directly on naïve CD8 cells, generating CTL with enhanced granzyme B expression. In vivo, IL-27 has been reported to augment CTL activity, inhibit tumor growth, and induce complete regression of primary and metastatic neuroblastoma tumors. It has been reported that IL-27 is critically involved in the induction of inflammation in rheumatoid arthritis.

Special 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.

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
Detection enzyme. Pre-titrated Avidin-HRP

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

Mouse IL-27 ELISA Ready-SET-Go!®

Catalog Number: 88-7274
Also known as: Interleukin-27
RUO: For Research Use Only. Not for use in diagnostic procedures.

Substrate Solution. Tetramethylbenzidine (TMB) Substrate Solution
Certificate of Analysis. Lot-specific instructions for dilution of antibodies and standards
96 Well Plate. Corning Costar 9018 (included with product Cat. #'s ending in suffixes -22, -44, -76, -86)

References

Fujita H, Teng A, Nozawa R, Takamoto-Matsui Y, Katagiri-Matsumura H, Ikezawa Z, Ishii Y. Production of both IL-27 and IFN-gamma after the treatment with a ligand for invariant NK T cells is responsible for the suppression of Th2 response and allergic inflammation in a mouse experimental asthma model. J Immunol. 2009 Jul 1;183(1):254-60.

Stumhofer JS, Hunter CA. Advances in understanding the anti-inflammatory properties of IL-27. Immunol Lett. 2008 May 15;117(2):123-30.

Pflanz S, Timans JC, Cheung J, Rosales R, Kanzler H, Gilbert J, Hibbert L, Churakova T, Travis M, Vaisberg E, Blumenschein WM, Mattson JD, Wagner JL, To W, Zurawski S, McClanahan TK, Gorman DM, Bazan JF, de Waal Malefyt R, Rennick D, Kastelein RA. IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naïve CD4(+) T cells. Immunity. 2002 Jun;16(6):779-90.

Devergne O, Coulomb-L'Herminé A, Capel F, Moussa M, Capron F. Expression of Epstein-Barr virus-induced gene 3, an interleukin-12 p40-related molecule, throughout human pregnancy: involvement of syncytiotrophoblasts and extravillous trophoblasts. Am J Pathol. 2001 Nov;159(5):1763-76.

Related Products

14-7273 Anti-Mouse EBI3 (IL-27 subunit) Purified (DNT27)
14-8271 Mouse IL-27 Recombinant Protein
34-8271 Mouse IL-27 Recombinant Protein Carrier-Free

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

eBioscience
An Affymetrix Company

Enzyme Linked Immunosorbent Assay (ELISA)

Research Use Only

Protocol: ELISA Ready-Set-Go!

The following protocol is a general guideline for the Ready-SET-Go! Sets

Materials Provided

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

Other Materials Needed

- Buffers*
  - Wash Buffer: 1x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, Cat. No. 00-0400)
  - Stop Solution: 1M H_3PO_4 (recommended) or 2N H_2SO_4
- Pipettes
- Refrigerator & frost-free -20°C freezer
- 96-well plate (Corning Costar 9018 or NUNC Maxisorp®)
  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 (Cat. No. 44-2404) 96-well plates
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer (highly recommended)

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.

Time Requirements

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

Experimental Procedure

1. Coat Corning Costar 9018 (or Nunc Maxisorp®) ELISA plate with 100 µL/well of capture antibody in Coating Buffer (dilute as noted on C of A, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 3 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. Optional: Aspirate and wash at least once with Wash Buffer.
5. Using 1X Assay Diluent*, dilute standards as noted on the C of A to prepare the top concentration of the standard. Add 100 µL/well of top standard concentration to the appropriate

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com •
info@ebioscience.com

eBioscience
An Affymetrix Company

Enzyme Linked Immunosorbent Assay (ELISA)

Research Use Only

wells. Perform 2-fold serial dilutions of the top standards to make the standard curve for a total of 8 points. Add 100 µL/well of your samples to the appropriate wells. 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 3-5 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 3-5 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 5-7 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.

NOTES:

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

*****The number of washes in the protocol was adapted to an automatic plate washer. This can be decreased when using other methods but should be tested empirically. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes.***

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com • info@ebioscience.com

eBioscience
An Affymetrix Company

Enzyme Linked Immunosorbent Assay (ELISA)

Research Use Only

Quick Guide: Standard Calibration

The following table indicates the protein standard contained in 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-8	1	1.8	180	89/520
hIL-10	1	0.8	4	93/722
hIL-12	1	0.8	8	95/544
hIL-17A	1	0.9	9000	01/420
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 being careful when washing and pipetting
	3. Contaminated substrate	3. Substrate should be colorless. Replace
	4. Incorrect dilutions, e.g., conjugate concentration was too high	4. Repeat using correct dilutions
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 using 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 making no enzyme inhibitor is present in any buffers.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com •
info@ebioscience.com

eBioscience
An Affymetrix Company

Enzyme Linked Immunosorbent Assay (ELISA)

Research Use Only

	4. Coated capture antibody in Assay Diluent rather than Coating Buffer	4. Repeat ELISA using Coating Buffer contained in the set as the diluent for the capture antibody.
C. Very weak signal	1. Improper and inefficient washing	1. Make sure washing procedure is done correctly, with a soak time.
	2. Incorrect dilutions of standard	2. Follow recommendations of standard preparation exactly as written on the C of A
	3. Insufficient incubation time	3. Repeat ELISA following the protocol carefully for each step
	4. Incorrect storage of reagents	4. Store reagents at the correct temperature asn indicated on the Technical Data Sheet. Freezing certain components will severely impact results. Do not re-use the standards.
	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 C of A. Edge effects can be avoided by moving samples and standards in from the edge of the plate.
	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. Reagents have expired	4. Order a new Ready-Set-Go ELISA.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • www.ebioscience.com •
info@ebioscience.com