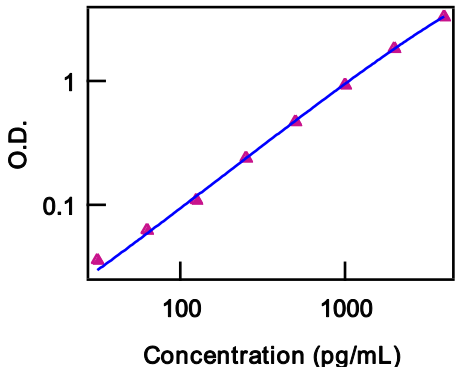
Mouse IL-28B (IFN lambda-3) ELISA Ready-SET-Go!®

Catalog Number: 88-7284
Also known as: Interleukin-28B
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Mouse IL-28 ELISA Ready-SET-Go!®

Product Information

Contents: Mouse IL-28B (IFN lambda-3) ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7284
Sensitivity: 32 pg/mL
Standard Curve Range: 32 - 4000 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-28 ELISA set contains all of the necessary reagents for the quantitative performance of enzyme-linked immunosorbent assays. It has been optimized for the accurate and precise measurement of IL-28 protein levels in serum, and tissue culture supernatant samples. The antibodies in this set were produced against and characterized with recombinant mouse IL-28B/ IFN lambda 3 and have not been evaluated for detection of mouse IL-28A/ IFN lambda 2. Cross-reactivity is probable, due to the high sequence homology between IL-28A and IL-28B.

IL-28 belongs to the IFN lambda family, a novel family of cytokines within the IL-10 superfamily. The three members of this family are IL-29 (IFN lambda 1), IL-28A (IFN lambda 2), and IL-28B (IFN lambda 3), and are also known as the type III Interferons. An active IL-29 gene is absent in mice, and sequence homology between mouse IL-28A and B is 97%. Expression of these cytokines has been observed in a wide variety of cells in response to viral infection and in response to TLR3 or TLR9 ligands.

The IFN lambda family signals through a heterodimeric receptor of which one subunit, IL-10R2, is shared with other members of the superfamily. The second subunit, IFN lambda R1 or IL-28R alpha, is unique to the IFN lambdas. Signaling occurs through the Jak/STAT pathway in a similar manner to signaling by the type I IFNs (IFN alpha/beta) and activates many of the same genes despite low sequence homology between the cytokines and receptors in the two families. Both IFN families display antiviral, antitumor, and antiproliferative effects, making the IFN lambdas potential alternatives to IFN-alpha for anticancer and antiviral therapies. Unlike the type I IFNs, which are able to stimulate most cells, response to IFN lambda stimulation appears to be limited to dendritic, epithelial, and some tumor cells. Another notable difference is the ability of the IFN lambda stimulation to drive dendritic cells towards the production of $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T-cells, suggesting a possible immunoregulatory role.

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

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

Mouse IL-28B (IFN lambda-3) ELISA Ready-SET-Go!®

Catalog Number: 88-7284
Also known as: Interleukin-28B
RUO: For Research Use Only. Not for use in diagnostic procedures.

Assay Diluent. 5X concentrated
Detection Enzyme. Pre-titrated Avidin-HRP
Substrate Solution. Tetramethylbenzidine (TMB) Solution
Certificate of Analysis. Lot-specific instructions for the 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-28/ IFN-λ in serum, plasma, and tissue culture supernatant samples.

Applications Tested
This assay was validated for the detection of endogenous protein with tissue culture supernatant collected from a culture of balb/c peritoneally elicited macrophages stimulated with CpG. No detectable levels of IL-28 were observed in unstimulated cells. Low amounts of non-specific interactions have been observed between CpG stimulants and the components of this set. It is recommended that any samples treated with CpG be incubated for 1 hour at room temperature with 1 µl of DNAse I per 100 µl of sample prior to assay.

This assay has been evaluated for specificity with a panel of 72 recombinant cytokines at 100 ng/ml. No cross-reactivity has been observed.

References
Ank N, Iversen M, Bartholdy C, Staeheli P, Hartmann R, Jensen U, Dagnaes-Hansen F, Thomsen A, Chen Z, Haugen H, Klucher K, and Paludan S. An important role for type III Interferon (IFN-lambda/IL-28) in TLR-induced antiviral activity. J Immuno. 2008; 180: 2474-85.

Ank N, West H, Bartholdy C, Eriksson K, Thomsen A, and Paludan S. Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. J Virol. 2006 May; 4501-9.

Commins S, Steinke JW, Borish L. The extended IL-10 superfamily: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29. J Allergy Clin Immunol. 2008 May; 121(5): 1108-11.

Related Products
14-8281 Mouse IL-28B (IFN lambda 3) Recombinant Protein
14-8288 Human IL-28B (IFN lambda 3) Recombinant Protein
14-8299 Human IL-29 (IFN lambda 1) Recombinant Protein
88-7296 Human IL-29 (IFN lambda 1) ELISA Ready-SET-Go!®

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