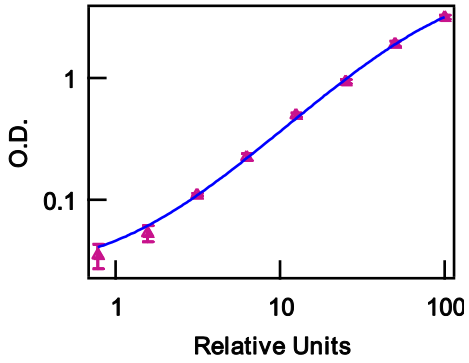
Human IL-35 ELISA Ready-SET-Go!

Catalog Number: 88-7357
Also known as: Interleukin-35, p35/EBI3
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Human IL-35 ELISA Ready-SET-Go!

Product Information

Contents: Human IL-35 ELISA Ready-SET-Go!
REF **Catalog Number:** 88-7357
Standard Curve Range: 1-100 Relative Units

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 Human IL-35 Ready-SET-Go! ELISA Set contains the necessary reagents, standards, buffers and diluents for performing quantitative enzyme-linked immunosorbent assays (ELISA). This ELISA set is specifically engineered for precise measurement of relative protein levels from samples including serum, plasma, cell lysates, and supernatants from cell cultures.The use of an EBI3-specific capture antibody and a p35-specific detection antibody renders this sandwich ELISA exquisitely specific for human IL-35.

Interleukin-35 (IL-35) is the most recently discovered member of the IL-12 subfamily of cytokines and is a heterodimer of the subunits EBI3 and p35. EBI3 is homologous to p40, and can be secreted independently as a monomer or homodimer, or along with p28 as the IL-27 cytokine. The p35 subunit was initially identified in association with p40 as the IL-12 cytokine, although the independent regulation of p40 and p35 expression led to speculation that these subunits could potentially associate with other proteins. This possibility was confirmed by the discovery of secreted EBI3 and p35 heterodimers in co-transfected cells.

Since its identification, the biology of the IL-35 heterodimer has remained incompletely understood, although it is clearly unique among the IL-12 family in expression and function. Early functional experiments demonstrated its ability to induce expansion and IL-10 production in CD4+ CD25+ Foxp3+ regulatory T cells and suppress the proliferation of Th17 cells, suggesting a role in immune regulation. This was further supported by the detection of IL-35 in supernatant from mouse regulatory, but not effector, T cell cultures by coprecipitation of the EBI3 and p35 subunits. However, attempts to produce similar results in natural, Foxp3+ human Treg were unsuccessful, suggesting that IL-35 had distinct functions in human and mouse biology. Later studies contradicted this by demonstrating that that inducible Treg, which are derived from mature effector T cells under certain stimulatory conditions, were capable of producing IL-35 after exposure to dendritic cells co-cultured with human rhinovirus (R-DC), and that the secreted IL-35 contributed to their immunosuppresant function. Additional research supported the findings that inducible Treg were a source of IL-35 and also indicated that IL-35 may play a role in the conversion of effector T cells to a suppressive phenotype. These findings provide further evidence that IL-35 functions mainly as an immunosuppressive cytokine.

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Components
Capture Antibody. Pre-titrated, purified antibody
Detection Antibody. Pre-titrated, biotin-conjugated antibody
Standard. Recombinant protein for generating standard curve and calibrating samples
Coating Buffer. 10X PBS ELISA Coating Buffer
Assay Diluent. 5X Concentrated
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, -76, and -86)

Applications Reported
This ELISA set is for thedetection of relative levels of human IL-35 in serum, plasma, and tissue culture supernatant samples.

Applications Tested
This assay has been validated for the detection of endogenous human IL-35 with supernatant collected from T cells co-cultured with rhinovirus-activated dendritic cells.

This assay was evaluated for specificity on a panel of 72 recombinant cytokines at 100 ng/mL. No significant crossreactivity was observed, including to human and mouse IL-12 and IL-27 as well as EBI3 alone.

References
Seyerl M, Kirchberger S, Majdic O, Seipelt J, Jindra C, Schrauf C, Stockl J. Human rhinoviruses induce IL-35-producing Treg via induction of B7-H1 (CD274) and sialoadhesin (CD169) on DC. Eur J Immunol. 2010 Feb;40(2):321-9.

Bardel E, Larousserie F, Charlot-Rabiega P, Coulomb-L'Hermine A, Devergne O. Human CD4+ CD25+ Foxp3+ regulatory T cells do not constitutively express IL-35. J Immunol 2008 Nov 15;181(10):6898-905.

Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature. 2007 Nov 22;450(7169):566-9.

Niedbala W, Wei XQ, Cai B, Hueber AJ, Leung BP, McInnes IB, Liew FY. IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells. Eur J Immunol. 2007 Nov;37(11):3021-9.

Devergne O, Birkenbach M, Kieff E. Epstein-Barr virus-induced gene 3 and the p35 subunit of interleukin 12 form a novel heterodimeric hematopoietin. Proc Natl Acad Sci USA. 1997 Oct 28;94(22):12041-6

Related Products
00-0400 ELISA Wash Buffer - 10 x 1L Packets
00-4201 1X TMB ELISA Substrate Solution
00-4202 ELISA Diluent Solution (5X)
88-7126 Human IL-12 p70 ELISA Ready-SET-Go!®
88-7278 Human IL-27 ELISA Ready-SET-Go!®

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Enzyme Linked Immunosorbent Assay (ELISA)

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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

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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.***

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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.

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	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.

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