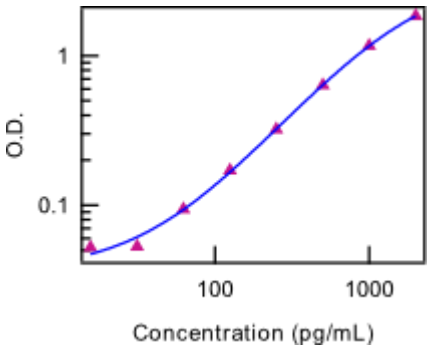
Human IL-23 ELISA Ready-SET-Go!® Set

Catalog Number: 88-7237
Also known as: Interleukin-23, IL23, p40, p19
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Human IL-23 ELISA Ready-SET-Go!®

Product Information

Contents: Human IL-23 ELISA Ready-SET-Go!® Set
REF **Catalog Number:** 88-7237
Sensitivity: 15 pg/mL
Standard Curve Range: 15 pg/mL - 2.0 ng/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
Caution, contains Azide

Description

This Human IL-23 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 accurate and precise measurement of human IL-23 protein levels from samples including serum, plasma, and supernatants from cell cultures. Interference with CpG oligodeoxynucleotides (or CpG ODN) has been observed with this ELISA kit. An improved sample diluent has been included that minimizes this effect; however, controls should still be added when utilizing the CpG compound in this assay. The assay demonstrates parallelism in measuring recombinant and native human IL-23 proteins with a standard curve range of 15 pg/ml to 2,000 pg/ml, and assay sensitivity below 15 pg/ml. The assay has been validated by specific detection of significant levels of native human IL-23 protein in supernatants from a variety of different activated dendritic cell populations. The use of a p19-specific capture antibody and a p40-specific detection antibody renders this sandwich ELISA exquisitely specific for human IL-23. IL-12 p40 monomer and IL-12 p70 were run in the assay at 200 ng/ml with no interference or cross-reactivity observed. A panel of 20 unrelated cytokines was also run in the IL-23 ELISA at 100 ng/ml with no cross reactivity observed.

IL-23 is a heterodimeric cytokine composed of the p40 subunit of IL-12 disulfide-linked with a protein p19. p19, like p35 of IL-12, is biologically inactive by itself. IL-23 interacts with IL-12Rbeta1 and an additional, novel beta2-like receptor subunit with STAT4 binding domain, termed IL-23R. IL-23 is secreted by activated mouse and human dendritic cells. Biological activities of mouse IL-23 are distinct from those of mouse IL-12. Mouse IL-23 was found not to induce significant amounts of IFN-g. Mouse IL-23 does induce strong proliferation of memory T cells (but not naïve T cells), whereas IL-12 has no effect on memory cells. Additionally, mouse IL-23 (but not IL-12) can activate mouse memory T cells to produce the proinflammatory cytokine IL-17. Human IL-23 has biological properties which are less distinct from human IL-12; human IL-23 induces proliferation of memory T cells and induces moderate levels of IFN-g production by naïve and memory T cells, as compared to IL-12. IL-23-dependent, IL-17-producing CD4+ T cells (Th-17 cells) have been identified as a unique subset of Th cells that develops along a pathway that is distinct from the Th1- and Th2- cell differentiation pathways. The hallmark effector molecules of Th1 and Th2 cells, e.g., IFN-g and IL-4, have each been found to negatively regulate the generation of these Th-17 cells. More recently, de novo differentiation of Th-17 cells in the absence of IL-23 has been demonstrated by treatment of naïve CD4 cells with

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

Human IL-23 ELISA Ready-SET-Go!® Set

Catalog Number: 88-7237
Also known as: Interleukin-23, IL23, p40, p19
RUO: For Research Use Only. Not for use in diagnostic procedures.

TGF-β1 and IL-6.

Components
Capture Antibody. Pre-titrated, purified antibody
Detection Antibody. Pre-titrated, biotin-conjugated antibody
Standard. Recombinant cytokine for generating standard curve and calibrating samples
ELISA/ELISPOT Coating Buffer Powder. This Ready-Set-Go! ELISA Set may contain ELISA/ELISPOT Coating Buffer Powder (Reconstitute to 1L with dH20 and filter (0.22 uM)) or 10X PBS ELISA Coating Buffer (Dilute 1 part 10X Buffer into 9 parts dH20).
Assay Diluent. 5X concentrated
Sample Diluent A. 12 ml of a 1X solution per plate
Detection enzyme. Pre-titrated Avidin-HRP
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
Goyarts E, Matsui M, Mammone T, Bender AM, Wagner JA, Maes D, Granstein RD. Norepinephrine modulates human dendritic cell activation by altering cytokine release. Exp Dermatol. 2008 Mar;17(3):188-96. (RSG ELISA kit, **TC supernatant**, PubMed)

Enstrom A, Onore C, Hertz-Picciotto I, Hansen R, Croen L, Van de WaterJ, Ashwood P. Detection of IL-17 and IL-23 in Plasma Samples of Children with Autism. American Journal of Biochemistry and Biotechnology 4 (2): 114-120, 2008. (RSG ELISA kit, **plasma**)

Iwakura Y, Ishigame H. The IL-23/IL-17 axis in inflammation. J Clin Invest. 2006 May;116(5):1218-22.

Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity. 2006 Feb;24(2):179-89.

Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. Interleukin 17–producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005 Nov;6(11):1123-32.

Brombacher F, Kastelein RA, Alber G. Novel IL-12 family members shed light on the orchestration of Th1 responses. Trends Immunol. 2003 Apr;24(4):207-12.

Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, Gurney AL. IL-23 promotes a distinct CD4 T cell activation state chaacterized by the production of IL-17. J. J Biol Chem. 2003 Jan 17;278(3):1910-4.

Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, Zonin F, Vaisberg E, Churakova T, Liu M, Gorman D, Wagner J, Zurawski S, Liu Y, Abrams JS, Moore KW, Rennick D, de Waal-Malefyt R, Hannum C, Bazan JF, Kastelein RA. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity. 2000 Nov;13(5):715-25.

Related Products
14-8348 Human TGF beta 1 Recombinant Protein
34-8129 Human IL-12 p70 Recombinant Protein Carrier-Free
34-8239 Human IL-23 Recombinant Protein Carrier-Free
39-8239 Human IL-23 Single-Use ELISA RSG Standard
88-7126 Human IL-12 p70 ELISA Ready-SET-Go!®

Human IL-23 ELISA Ready-SET-Go!® Set

Catalog Number: 88-7237
Also known as: Interleukin-23, IL23, p40, p19
RUO: For Research Use Only. Not for use in diagnostic procedures.

88-7879 Human IL-12 p70 ELISPOT Ready-SET-Go!®

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

full spectrum cell analysis
eBioscience™

TDS Protocol

Research Use Only

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

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

TDS Protocol

Research Use Only
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 A, 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, diluting them at least 2-fold in Sample Diluent A**. 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.**

****NOTE: Interference by CpG oligodeoxynucleotides (or CpG ODN) has been reported in this ELISA. Dilution of samples containing CpG compounds at least 2-fold in Sample Diluent A will minimize this effect. However, appropriate controls should still be added whenever utilizing CpG compounds. Samples not containing CpG may be tested undiluted in this ELISA.**

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

TDS Protocol

Research Use Only

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

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

TDS Protocol

Research Use Only

	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
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

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