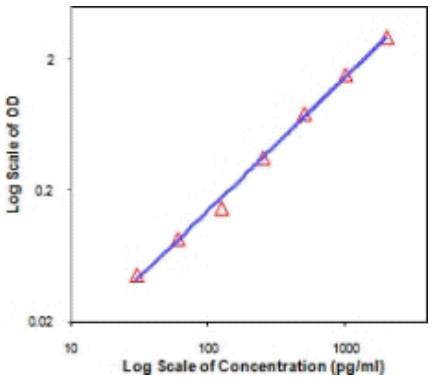
Human IL-17AF (heterodimer) ELISA Ready-SET-Go!®

Catalog Number: 88-7117
Also Known As: Interleukin-IL-17AF, IL17AF
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Human IL-17AF (heterodimer) ELISA Ready-SET-Go!®

Product Information

Contents: Human IL-17AF (heterodimer) ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7117
Sensitivity: 30 pg/mL
Standard Curve Range: 30-4000 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
Caution, contains Azide

Description
This human IL-17AF ELISA Ready-SET-Go! Reagent set (with or without high-affinity binding 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 human IL-17AF protein levels from serum, plasma and cell culture supernatants. This assay demonstrates parallelism in measuring recombinant and native human IL-17AF proteins and a standard curve range of 30 to 4000 pg/ml. Minimal crossreactivity to the IL-17A homodimer is observed (2%) and with no crossreactivity to IL-17F when spiked in excess (100ng/ml).

IL-17A and IL-17F are members of the IL-17 family and signature Th17 cytokines. Of the six IL-17 family members, IL-17F and IL-17A share the strongest homology (50% amino acid identity) and the two genes are located in the same chromosomal region. Although both IL-17A and IL-17F were originally found to be produced as disulfide-linked homodimers, recent studies have confirmed the production of IL-17AF heterodimers in in vitro-differentiated and polarized Th17 cells. Activated human CD4+ T cells in culture were found to secrete the IL-17F homodimer at 10-fold higher levels than the IL-17A homodimer, suggesting that the majority of the IL-17A protein expressed exists in the form of the IL-17AF heterodimer. Studies of Th17-polarized mouse splenocytes also indicate that the majority of IL-17 produced is complexed as the IL-17AF heterodimer. IL-17F and IL-17A have been observed in tissue samples from various autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, psoriasis, inflammatory bowel disease, and asthma.

Components
Capture Antibody. Pre-titrated, purified antibody
Detection Antibody. Pre-titrated, biotin-conjugated antibody
Standard. Recombinant cytokine for generating standard curve and calibrating samples
Assay Diluent. 5X concentrated
Wash Buffer. 10X concentrated (100 ml of 10X per plate)
Detection enzyme. Pre-titrated Avidin-HRP
Substrate Solution. Tetramethylbenzidine (TMB) Substrate Solution
Stop Solution. 5 mls of 1X solution per plate
Certificate of Analysis. Lot-specific instructions for dilution of antibodies and standards

References
Chang SH, Dong C. A novel heterodimeric cytokine consisting of IL-17 and IL-17F regulates inflammatory responses. Cell Res. 2007 May;17 (5):435-40.

Wright JF, Guo Y, Quazi A, Luxenberg DP, Bennett F, Ross JF, Qiu Y, Whitters MJ, Tomkinson KN, Dunussi-Joannopoulos K, Carreno BM, Collins M, Wolfman NM. Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. J Biol Chem. 2007 May 4;282(18):13447-55.

Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med. 2006 Oct 2;203(10):2271-9.

Related Products

13-7179 Anti-Human IL-17A Biotin (eBio64DEC17)
14-7178 Anti-Human IL-17A Purified (eBio64CAP17)
14-8178 Human IL-17AF Recombinant Protein
34-8178 Human IL-17AF Recombinant Protein Carrier-Free
88-7176 Human IL-17A (homodimer) ELISA Ready-SET-Go!®

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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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