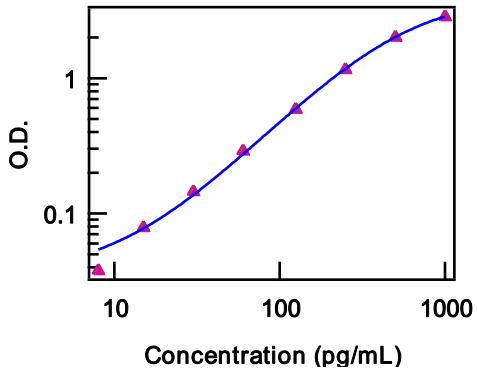
# Mouse IL-22 ELISA Ready-SET-Go!®

**Catalog Number:** 88-7422
**Also known as:** Interleukin-22
**RUO: For Research Use Only. Not for use in diagnostic procedures.**




Standard curve of Mouse IL-22 ELISA Ready-SET-Go!®

## Product Information

**Contents:** Mouse IL-22 ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7422
**Sensitivity:** 8 pg/mL
**Standard Curve Range:** 8 - 1000 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-22 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 mouse IL-22 protein levels from samples including serum, and supernatants from cell cultures. The assay demonstrates parallelism in measuring recombinant and native mouse IL-22 proteins and a standard curve range of 8 to 1000 pg/ml. Human IL-22 receptor at levels above 4ng/ml and IL-22 binding protein at level above 3ng/ml interfere with the assay, which suggest that the assay mainly detect the free IL-22.

IL-22 is a 20 kDa member of the IL-10 cytokine family that is secreted primarily by Th17 cells. IL-23 and IL-6 can directly stimulate naive T cell to produce IL-22. Some NK subset cells, so called NK22, have also been shown to produce IL-22 in respose to IL-23 stimulation. In in vitro Th17 cultures, induction of IL-22 expression is greater in response to IL-23 than IL-6 or TGF-ß, suggesting that this cytokine may be secreted by more fully differentiated Th17 cells in vivo. Recently it is reported that self antigen selected Th17 cells in thymus are recruited to the lung, gut and liver. These cells secrete IL-22 in response to self antigen and mediated host protection during inflammation. Elevated IL-22 serum levels have also been reported in the mouse acute hepatitis model.

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

Marks BR, Nowyhed HN, Choi JY, Poholek AC, Odegard JM, Flavell RA, Craft J. Thymic self-reactivity selects natural interleukin 17-producing T cells that can regulate peripheral inflammation. Nat Immunol. 2009 Oct;10(10):1047-9.

Harper EG, Guo C, Rizzo H, Lillis JV, Kurtz SE, Skorcheva I, Purdy D, Fitch E, Iordanov M, Blauvelt A.Th17 Cytokines Stimulate CCL20 Expression in Keratinocytes In Vitro and In Vivo: Implications for Psoriasis Pathogenesis. J Invest Dermatol. 2009 Sep;129(9):2175-83.

Hughes T, Becknell B, McClory S, Briercheck E, Freud AG, Zhang X, Mao H, Nuovo G, Yu J, Caligiuri MA. Stage three immature human natural killer cells found in secondary lymphoid tissue constitutively and selectively express the TH17 cytokine interleukin-22. Blood. 2009 May;87(5):451-4.

Aujla SJ, Kolls JK. IL-22: A critical mediator in mucosal host defense. J Mol Med.2009 May;87(5):451-4.

Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of T(H)17 cells. Nature. 2008 Jun 19;453(7198):1051-7.

**Related Products**

12-7221 Anti-Mouse IL-22 PE (1H8PWSR)
14-8221 Mouse IL-22 Recombinant Protein
34-8221 Mouse IL-22 Recombinant Protein Carrier-Free
88-7522 Human IL-22 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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