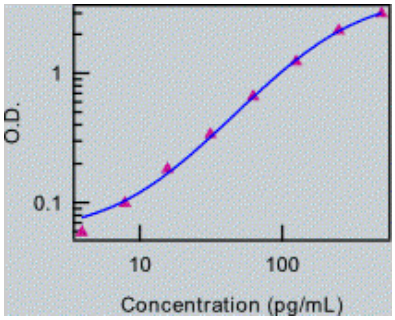
Mouse IL-15/IL-15R Complex ELISA Ready-SET-Go!®

Catalog Number: 88-7215
Also Known As:IL-15 Receptor complex with IL-15
RUO: For Research Use Only. Not for use in diagnostic procedures.



Standard curve of Mouse IL-15/IL-15R Complex ELISA Ready-SET-Go!®

Product Information

Contents: Mouse IL-15/IL-15R Complex ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7215
Sensitivity: 4 pg/mL
Standard Curve Range: 4 - 500 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 Interleukin-15/Interleukin-15 Receptor (IL-15/IL-15R) ELISA set contains all of the necessary reagents for the performance of quantitative enzyme-linked immunosorbent assays. It has been optimized for the accurate and precise measurement of IL-15/IL-15R protein levels from serum, and tissue culture supernatant samples. Recombinant mouse IL-15 has a cross-reactivity of 2% in this assay when spiked in excess (100 ng/ml). The specificity of this ELISA has been confirmed with bone-marrow derived dendritic cells from IL-15 knock-out mice; Tissue culture supernatant from these cells following lipopolysaccharide (LPS) stimulation was negative for the presence of IL-15/IL-15R complex while wild type animals reveal more than 40pg/ml.

Interleukin-15 (IL-15) is a 14 kDa proinflammatory protein that has been shown to play a role in the activation of neutrophils, dendritic cells, and macrophages, and is essential to the development and survival of NK cells and CD8 T-cells. IL-15 activates Jak/Stat pathways by signaling through a heterotrimeric receptor. Two of the subunits of this receptor, β and γ, are shared with the IL-2 receptor, while the alpha subunit is unique to IL-15.

Despite the expression of IL-15 mRNA in many cell types, the secreted protein is rarely detectable in biological samples. Recent research suggests that IL-15 is retained inside the cell and is only secreted in complex with its unique receptor, IL-15R alpha. This chaperoning is required from the ER to Golgi through to secretion. The cytokine/receptor complex may stay bound to the cell surface, where it can be trans-presented to cells expressing the beta gamma subunits of the receptor, or may be secreted in a soluble form that lacks the transmembrane domain of the receptor. The soluble IL-15/IL-15R complex is produced by mouse dendritic cells, fibroblasts, and macrophages, and demonstrates a 10-100-fold increase in agonistic activity over IL-15 alone.

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) Solution
Certificate of Analysis. Lot-specific instructions for the dilutions of antibodies and standards
96-Well Plates. Corning Costar Flat-bottom (included with Cat. #'s ending in -22 and -86)

Applications Reported
This ELISA set is for the quantitative detection of mouse IL-15/IL-15R complex in serum, plasma, and tissue culture supernatant samples.

References
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Dafik L, Albertelli M, Stamnaes J, Sollid LM, Khosla C. Activation and inhibition of transglutaminase 2 in mice. PLoS One. 2012;7(2):e30642. (**RSG**, PubMed)

Khawam K, Giron-Michel J, Gu Y, Perier A, Giuliani M, Caignard A, Devocelle A, Ferrini S, Fabbi M, Charpentier B, Ludwig A, Chouaib S, Azzarone B, Eid P. Human renal cancer cells express a novel membrane-bound interleukin-15 that induces, in response to the soluble interleukin-15 receptor alpha chain, epithelial-to-mesenchymal transition. Cancer Res. 2009 Feb 15;69(4):1561-9.

Duitman EH, Orinska Z, Bulanova E, Paus R, Bulfone-Paus S. How a cytokine is chaperoned through the secretory pathway by complexing with its own receptor: lessons from interleukin-15 (IL-15)/IL-15 receptor alpha. Mol Cell Biol. 2008 Aug;28(15):4851-61

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Zhang H and Meadows GG. Exogenous IL-15 in combination with IL-15R alpha rescues natural killer cells from apoptosis induced by chronic alcohol consumption. Alcohol Clin Exp Res. 2009 Mar;33(3):419-27.

Related Products
14-8152 Mouse IL-15/IL-15R Complex Recombinant Protein

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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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