

## Mouse IL-23 ELISA Ready-SET-Go!<sup>®</sup> (Second generation assay)

**Catalog Number:** 88-7230

**Also Known As:** Interleukin-23, IL23, p40, p19

**RUO: For Research Use Only. Not for use in diagnostic procedures.**


### Product Information

**Contents:** Mouse IL-23 ELISA Ready-SET-Go!<sup>®</sup> (Second generation assay)

**REF** **Catalog Number:** 88-7230

**Sensitivity:** 8 pg/ml

**Standard Curve Range:** 1000 pg/ml - 8 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 Mouse IL-23 (p19/p40) ELISA Ready-SET-Go!<sup>®</sup> 'IS' ELISA Set (Improved sensitivity assay with 5B2 capture antibody) with high affinity binding ELISA plates contains the necessary reagents, buffers and diluents for performing quantitative, enzyme-linked immunosorbent assays (ELISA). The 5B2 antibody in combination with the C17.8 antibody yields an improved IL-23 sandwich ELISA with greater sensitivity than was achievable with previous assays. This ELISA reagent set is specifically engineered for accurate and precise measurement of mouse IL-23 protein levels from samples including serum, and supernatants from cell cultures. The assay demonstrates parallelism in measuring recombinant and native mouse IL-23 proteins with a standard curve range of 4 pg/ml to 500 pg/ml and assay sensitivity below 4 pg/ml. Native mouse IL-23 was detected in supernatants from bone marrow-derived, LPS-activated dendritic cells. The use of a p19-specific capture antibody and a p40-specific detection antibody renders this assay exquisitely specific for mouse IL-23. IL-12 p40 homodimer and IL-12 p70 were each run in the assay at 500 ng/ml with no interference or cross-reactivity observed. A panel of 20 unrelated cytokines was run in the IL-23 ELISA at 100 ng/ml with no cross reactivity observed; all values were at the limit of detection of the assay.

For measurement of total p40 protein levels, the Mouse IL-12/23 Total p40 ELISA Ready-SET-Go!<sup>®</sup> is available (88-7120).

### 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!<sup>®</sup> ELISA Set may contain ELISA/ELISPOT Coating Buffer Powder (Reconstitute to 1L with dH2O and filter (0.22 µm)) or 10X PBS ELISA Coating Buffer (Dilute 1 part 10X Buffer into 9 parts dH2O).

**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.** Corning Costar 9018 flat-bottom (included with product Cat. #'s ending in suffixes -22, -44, -76, -86)

### Related Products

14-7233 Anti-Mouse IL-23 p19 Purified (5B2)

34-8171 Mouse IL-17A Recombinant Protein Carrier-Free

34-8231 Mouse IL-23 Recombinant Protein Carrier-Free

34-8348 Human TGF beta 1 Recombinant Protein Carrier-Free

39-8231 Mouse IL-23 Single-Use ELISA RSG Standard

88-7344 Human/Mouse TGF beta 1 ELISA Ready-SET-Go!<sup>®</sup> (To Be Discontinued. Refer to 2nd Generation RSG Version: cat. 88-8350)

88-7371 Mouse IL-17A (homodimer) ELISA Ready-SET-Go!<sup>®</sup>

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## 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<sub>3</sub>PO<sub>4</sub> or 2N H<sub>2</sub>SO<sub>4</sub>

☐ 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 performance 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 6 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 6 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

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## TDS Protocol




### Research Use Only Experimental Procedure

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1. Coat Corning Costar 9018 ELISA plate with 100  $\mu$ l/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  $\mu$ l/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  $\mu$ l/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 1X Assay Diluent\*, dilute standards as noted on the Certificate of Analysis (C of A). Add 100  $\mu$ l/well of standard to the appropriate wells. Perform 2-fold serial dilutions of the top standards to make the standard curve. Add 100  $\mu$ l/well of your samples to the appropriate wells. 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  $\mu$ l/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  $\mu$ l/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  $\mu$ l/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50  $\mu$ l 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.**

#### Ready-SET-Go Cytokine ELISA Set Buffers:

-  Assay Diluent (5X concentrate): Dilute 1/5 in DI water.
-  Substrate Solution: Ready to use (1X); 100  $\mu$ l per well.
-  ELISA/ELISPOT Coating Buffer Powder: Reconstitute in 1L dH2O; filter (0.22  $\mu$ M).

## 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
	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

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## TDS Protocol

### Research Use Only

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