invitrogen PRODUCT INFORMATION SHEET

Mouse IL-1 beta Pro-form Uncoated ELISA

Enzyme-linked immunosorbent assay for quantitative detection of mouse IL-1 beta Pro-form

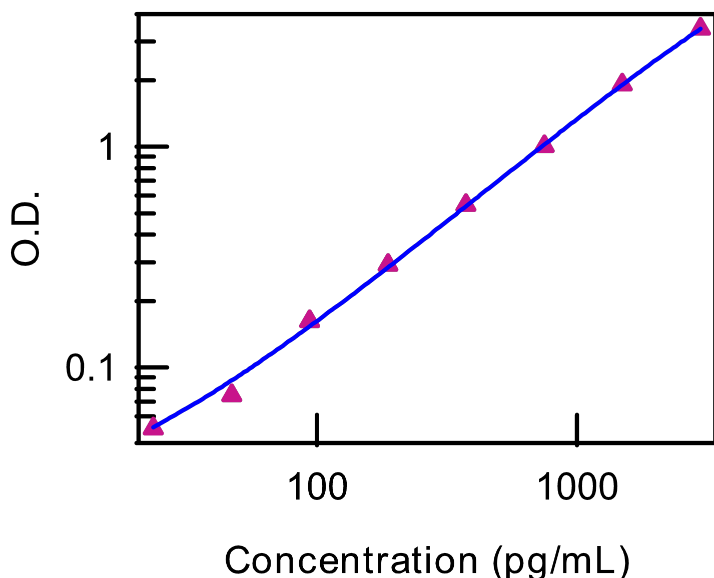
Catalog Number 88-8014

Pub. No. MAN0017524 **Rev.** A.0 (31)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Standard curve of mouse IL-1 beta Pro-form

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Product information

Symbol	Contents	Mouse IL-1 beta Pro-form Uncoated ELISA
REF	Catalog number	88-8014
—	Sensitivity	25 pg/mL
—	Standard curve range	25-3000 pg/mL
	Temperature limitation	Store at 2–8°C
LOT	Batch code	Refer to vial
	Use by	Refer to box label
	Caution	Contains preservatives

Description

This Mouse IL-1 beta Pro-form Uncoated ELISA 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 mouse IL-1 beta Pro-form protein levels from samples including serum, plasma, cell lysates, and supernatants from cell cultures.

Components of 2-plate format (2x96 tests)

- **Capture Antibody:** Pre-titrated, purified anti-mouse IL-1 beta Pro-form antibody

  1 vial (100 µL) Capture Antibody Concentrate (250X)
- **Detection Antibody:** Pre-titrated, biotin-conjugated anti-mouse IL-1 beta Pro-form antibody

  1 vial (100 µL) Detection Antibody Concentrate (250X)
- **Standard:** Recombinant mouse IL-1 beta Pro-form for generating standard curve and calibrating samples

  2 vials mouse IL-1 beta Pro-form Standard (lyophilized): 3000 pg/mL upon reconstitution
- **Coating Buffer**

  1 vial (2.5 ml) Phosphate Buffered Saline Concentrate (PBS, 10X)
- **5X ELISA/ELISPOT Diluent**

  1 bottle (30 ml) Diluent Concentrate (5X)
- **Enzyme**

  1 vial (100 µL) pre-titrated Avidin-HRP Concentrate (250X)
- **Substrate Solution:** Tetramethylbenzidine (TMB) Substrate Solution

  1 bottle (20 ml)
- **96-well plates:** Corning Costar 9018

  2 plates

Other materials needed

- Buffers
  - Wash Buffer: 1X PBS, 0.05% Tween™-20 or eBioscience™ Wash Buffer (20X) Cat. Nos. BMS408.0500 or 00-0400-46
  - Stop Solution: 1 M H_3PO_4 or 2 N H_2SO_4 or eBioscience™ Stop Solution Cat. Nos. BMS409.0100, SS03, SS03100, or SS04
- Pipettes and pipettors
- Refrigerator
- 96-well plate (Corning™ Costar™ 9018)

  Note: The use of ELISA plates that 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
- *(Optional)* Microplate shaker

Note: To ensure optimal results from using this kit, use only the components included in the set. Exchanging of components is not recommended because a change in performance may occur.

Stability

This kit is guaranteed to perform as defined if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents. Expiration date is indicated on the box label.

Storage instructions for kit reagents

Store at 2-8°C.

Reagent preparation

Note: If crystals form in the buffer concentrate, warm them gently until they completely dissolve.

- **Coating Buffer (1X)**

  Make a 1:10 dilution of PBS (10X) in deionized water.
- **Capture Antibody**

  Dilute capture antibody (250X) 1:250 in Coating Buffer (1X).
- **5X ELISA/ELISPOT Diluent**

  Dilute Diluent Concentrate (5X) 1:5 in deionized water.
- **Standard**

  Reconstitute mouse IL-1 beta Pro-form standard by addition of ELISA/ELISPOT Diluent (1X). Reconstitution volume is stated on the label of the standard vial. Allow the standard to reconstitute for 10–30 minutes. Swirl or mix gently to ensure complete and homogeneous solubilization (concentration of reconstituted standard = 3000 pg/mL).

  Mix well prior to making dilutions. The standard has to be used immediately after reconstitution and cannot be stored.
- **Detection Antibody**

  Dilute detection antibody (250X) 1:250 in ELISA/ELISPOT Diluent (1X).
- **Enzyme**

  Dilute HRP Concentrate (250X) 1:250 in ELISA/ELISPOT Diluent (1X).

Experimental procedure

Note: Shaking is recommended for all incubation steps.

Note: In case of incubation without shaking, the obtained O.D. values may be decreased. Nevertheless the results are still valid.

Note: Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.

1. Coat Corning™ Costar™ 9018 ELISA plate with 100 µL/well of capture antibody in Coating Buffer (dilute as noted in point 1 of Reagent preparation). 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. Block wells with 200 µL of ELISA/ELISPOT Diluent (1X). Incubate at room temperature for 1 hour.
4. Prepare Standard (see Reagent preparation).
5. Aspirate and wash at least once with Wash Buffer.
6. Perform 2-fold serial dilutions of the top standards to make the standard curve for a total of 8 points.

   For that add 100 µL of ELISA/ELISPOT Diluent (1X) to the wells leaving the first wells empty. Add 200 µL/well of top standard concentration to the first empty wells A1/A2. Transfer 100 µL of top standard from wells A1/A2 to wells B1/B2. Mix the contents of the wells B1 and B2 by repeated aspiration and ejection and transfer 100 µL to wells C1/C2. Take care not to scratch surface of the microwells. Continue this procedure 5 times.
7. Add 100 µL/well of samples to the appropriate wells.
8. Add 100 µL of ELISA/ELISPOT Diluent (1X) to the blank well.
9. Seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximum sensitivity).
10. Prepare the Detection Antibody (See Reagent preparation).
11. Aspirate and wash as in Step 2. Repeat for a total of 3-5 washes.

    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.
12. Add 100 µL/well diluted Detection Antibody to all wells.
13. Seal the plate and incubate at room temperature for 1 hour.
14. Prepare the Avidin-HRP (see Reagent preparation).
15. Aspirate and wash as in Step 2. Repeat for a total of 3-5 washes.

    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.
16. Add 100 µL/well of diluted Avidin-HRP.
17. Seal the plate and incubate at room temperature for 30 minutes.
18. Aspirate and wash as in Step 2, making sure to allow time for soaking for 1 to 2 minutes prior to aspiration. Repeat for a total of 5-7 washes.
19. Add 100 µL/well of 1X TMB Solution.
20. Incubate at room temperature for 15 minutes.
21. Add 100 µL/well of Stop Solution.
22. Read plate at 450 nm. If wavelength substraction is available, substract the values of 570 nm from those of 450 nm and analyze data.

ELISA troubleshooting guide

Problem	Possibility	Solution
High background	Improper and inefficient washing.	Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed.
	Cross contamination from other specimens or positive controls.	Repeat ELISA, be careful when washing and pipetting.
	Contaminated substrate.	Substrate should be colorless.
	Incorrect dilutions, e.g., conjugate concentration was too high.	Repeat test using correct dilutions; check with manufacturer.
No signal	Improper, low protein binding capacity plates were used.	Repeat ELISA, using recommended high binding capacity plates.
	Wrong substrate was used.	Repeat ELISA, use the correct substrate.
	Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity.	Repeat ELISA, make sure your system contains no enzyme inhibitor.
Very weak signal	Improper and inefficient washing.	Make sure washing procedure is done correctly.
	Incorrect dilutions of standard.	Follow recommendations of standard handling exactly as written in Reagent preparation and Experimental procedures.
	Insufficient incubation time.	Repeat ELISA, follow the protocol carefully for each step's incubation time.
	Incorrect storage of reagents.	Store reagents in the correct temperature, avoid freeze and thaw, avoid using the frost free freezer.
	Wrong filter in ELISA reader was used.	Use correct wavelength setting.
	Wrong plate used.	Use the recommended Corning™ Costar™ 9018 or Nunc™ MaxiSorp™ flat bottom 96-well plates.
Variation among replicates	Improper and inefficient washing.	Make sure washing procedure is done correctly.
	Poor mixing of samples.	Mix samples and reagents gently and equilibrate to proper temperature.
	Plates not clean.	Plates should be wiped on bottom before measuring absorbance.
	Improper, low binding capacity plates were used.	Use recommended high binding capacity plates.
	Reagents have expired.	Do not use if past expiration date.
Variation of kit performance	Different buffers, plates. Handling can strongly affect kit performance.	Use recommended buffers, plates, and kit components.

Customer and technical support

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  - Software, patches, and updates
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- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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