ABSbio™ Rat IL-1β ELISA Kit

Rat IL-1β Quantification (96 tests)

Introduction

Interleukin-1β (IL-1β) is a potent stimulator of bone resorption whose gene is mapped to 2q14, and has been implicated in the pathogenesis of high bone turnover and osteoporosis. ABSbio™ Rat IL-1β ELISA Kit provide a rapid, sensitive and reliable assay for the determination of Rat IL-1β in serum, plasma & cell culture media. The strip plate is coated with the antibody and pre-blocked to provide timesaving for high-throughput users. Rat IL-1β containing samples or standards and a biotinylated detection antibody directed against Rat IL-1β are added subsequently and then followed by washing step. Streptavidin-HRP was added and unbound conjugates were washed away. Finally, TMB was used to visualize HRP enzyme reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Rat IL-1β in samples. The kit is designed to quantitatively measure natural and/or recombinant Rat IL-1β within the range of **25–4000 pg/ml**. The kit is sufficient to assay Rat IL-1β in approximately 96 ELISA plate wells.

Kit Components (96 tests)

Rat IL-1β Standard:	1 vial	Assay Diluent:	30 mL	TMB Solution:	12 mL	Plate Sealer:	2	Detection Antibody:	1 vial
Streptavidin-HRP:	1 vial	Wash Solution:	30 mL	Stop Solution:	12 mL			Antibody coated plate:	1

Storage and Handling: Shipping on ice. Store standard and antibody at -20℃, other components at 2-8 ℃. Shelf Life: 6 months after receipt. Warm up plate to room temperature before use.

Reagent Preparation

- After taking out from 2-8℃ refrigerator, prewarm the kit 30 minutes at room temperature before using. Secure the desired numbers of coated wells in the holder.
- **Wash Solution:** 10x dilute Wash Solution with dH_2O to prepare 1x Wash Solution.
- **Detection Antibody:** Immediately before use, add 30 µl Assay Diluent into Detection Antibody vial to reconstitute Detection Antibody. Transfer 12 µl of the detection ab into 11 mL of Assay Diluent for one plate (for partial plate assay, adjust the volumes accordingly).
- **Streptavidin-HRP:** Immediately before use, add 60 µl of the Streptavidin-HRP conjugate into 11 mL of Assay Diluent for one plate (for partial plate assay, adjust the volumes accordingly).
- **Rat IL-1β Standard:** Add 1 mL of Assay Diluent into the standard vial to reconstitute standard. The aliquoted standard can be stored at -20℃ for up to 6 months. Transfer 4 µl of reconstitute standard into 1000 µl Assay Diluent to make the high standard concentration of 4000 pg/mL and vortex for 20 sec and allow it to sit for 5 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in Assay Diluent.

Procedure Guideline

1. Prepare sample dilutions (3 or 10 fold) in a clean 96-well plate with Assay Diluent or 1x PBS.
2. Set standard wells, testing sample and blank wells on the assay plate/strip. Transfer diluted **standard** 100 µl to standard wells, 100 µl **sample** to sample wells, 100 µl **Assay Diluent** only to blank wells. Assay should be run in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	4000pg/mL standard	4000pg/mL standard	Undiluted sample 1	Undiluted sample 1								
B	2000pg/mL	2000pg/mL	3-fold	3-fold								
C	1000pg/mL	1000pg/mL	Undiluted sample 2	Undiluted sample 2								
D	500pg/mL	500pg/mL	3-fold	3-fold								
E	250pg/mL	250pg/mL	Undiluted sample 3	Undiluted sample 3								
F	125pg/mL	125pg/mL	3-fold	3-fold								
G	62.5pg/mL	62.5pg/mL	Undiluted sample 4	Undiluted sample 4								
H	blank	blank	3-fold	3-fold								

3. Cover the plate with plate sealer and incubate at room temperature for 1-2 hour.
4. Decant as much liquid as possible, fill the wells with 250 µl wash solution, oscillate the plate on an oscillating shaker if available for 1 min, decant the wash solution and remove residual liquid with absorbent paper. Repeat wash three times.
5. Add 100 µl diluted **Detection ab** into each well, and cover the plate with plate sealer, incubate the plate at room temperature for 1 hr on an oscillating shaker.
6. Repeat washing step 4.
7. Add 100 µl of the prepared **Streptavidin-HRP** conjugate into each wells. Cover the plate with plate sealer and incubate at room temperature for 0.5 hour.
8. Repeat washing step 4.
9. Add 100 µl of **TMB Solution** to each well and incubate at room temperature for 10~30 minutes, or keep close monitoring on the developing process until desired developing blue color observed.

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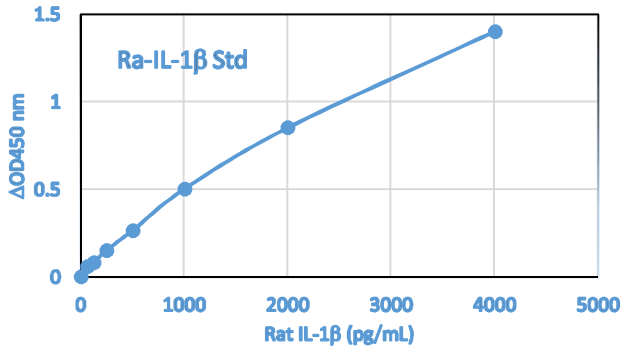
10. Add 100 µl of **Stop Solution** to each well to stop the reaction (the blue color change to yellow).
11. Read absorbance of the plate on a microplate reader at 450 nm within 15 min.
12. Average the duplicate OD readings for each standard, blank and sample. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-1β concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve



Quantification of Rat IL-1β.
Standard, Samples and detection ab were incubated at room temperature for 1-2 hours in the coated plate. For ELISA analysis using Rat IL-1β ELISA Kit (#Ra-IL-1β), the Streptavidin-HRP conjugate was incubated for another 0.5 hour at room temperature. Following three wash steps. The peroxidase was detected by using a soluble TMB solution for ELISA applications. The reaction was stopped using stop solution. Absorbance was read on a Molecular Devices SpectraMax® at 450 nm.

Always run your own standard curves for calculation of results. ***For research use only.***

Sample collection and storage

Store samples to be assayed within 24 hours at 2-8℃. For long-term storage, aliquot and freeze samples at -20℃.
Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20℃.
Cell culture media: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20℃.
Plasma: Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20℃.

Performance Data

Standard range 25- 4000 pg/mL	**Sensitivity 10 pg/mL**
Assay time 3 hours	**Validity Six months**
Coefficients of variation (CVs) for Std <10%;	**CVs for samples: <10%**

Cross-reactivity: Rat IL-1β 100%; Murine & Human IL-1β no significant cross reactivity.

Related Products:

Murine IL-1β ELISA Kit (#Mu-IL-1β)	**Human IL-1β ELISA Kit (#Hu-IL-1β)**
Equine IL-1β ELISA Kit (#Eq-IL-1β)	**Bovine IL-1β ELISA Kit (#Bo-IL-1β)**
Chicken IL-1β ELISA Kit (#Ch-IL-1β)	**Pig IL-1β ELISA Kit (#Pi-IL-1β)**

TROUBLE SHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure that all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with washing solution.