

## Mouse IL-1 beta Sandwich ELISA Kit Datasheet

For the quantitative detection of mouse IL-1 beta concentrations in serum, plasma, cell culture supernatants, cell lysates and tissue homogenates.

### General Information

|                           |                                    |
|---------------------------|------------------------------------|
| Catalogue Number          | KE10003                            |
| Product Name              | Mouse IL-1 beta Sandwich ELISA Kit |
| Species cross-reactivity  | Mouse                              |
| Range (calibration Range) | 7.8-500 pg/mL                      |
| Tested applications       | Quantification ELISA               |

### Database Links

|             |        |
|-------------|--------|
| Entrez Gene | 16176  |
| SwissProt   | P10749 |

### Kit Components & Storage

|   |           |   |
|---|-----------|---|
| Microplate - antibody coated 96-well microplate (8 well × 12 strips)                          | 1 plate   | <b>Unopened Kit:</b><br>Store at 2-8°C for 6 months or -20°C for 12 months.<br><br><b>Opened Kit:</b><br>All reagents stored at 2-8°C for 7 days.<br><br><b>Please use a new standard for each assay.</b> |
| Protein standard - 1000 pg/bottle; lyophilized*   | 2 bottles |   |
| Detection antibody, biotinylated (100X) - 120 µL/vial   | 1 vial    |   |
| Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial                                | 1 vial    |   |
| Sample Diluent PT 1-ec - 30 mL/bottle. For serum, plasma, cell lysates and tissue homogenates | 1 bottle  |   |
| Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants                          | 1 bottle  |   |
| Detection Diluent - 30 mL/bottle  | 1 bottle  |   |
| Wash Buffer Concentrate (20X) - 30 mL/bottle  | 1 bottle  |   |
| Extraction Reagent - 30 mL/bottle   | 1 bottle  |   |
| Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle   | 1 bottle  |   |
| Stop Solution - 12 mL/bottle  | 1 bottle  |   |
| Plate Cover Seals   | 3 pieces  |   |

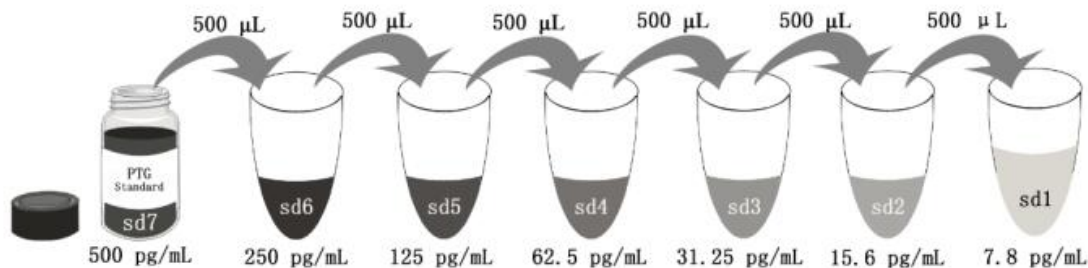
**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 1-ec is for protein standard, serum, plasma, cell lysates and tissue homogenates.

Sample Diluent PT 1-ef is for protein standard and cell culture supernatants.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 2 mL Sample Diluent PT 1-ec or PT 1-ef in standard. This reconstitution gives a stock solution of 500 pg/mL.



|   |         |        |        |        |        |        |        |
|---|---------|--------|--------|--------|--------|--------|--------|
| Add # µL of Standard diluted in the previous step | —       | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| # µL of Sample Diluent PT 1-ec or PT 1-ef         | 2000 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
|   | "sd7"   | "sd6"  | "sd5"  | "sd4"  | "sd3"  | "sd2"  | "sd1"  |

## Product Description

KE10003 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL1-beta ELISA kit is to be used to detect and quantify protein levels of endogenous IL1-beta. The assay recognizes mouse IL1-beta. An antibody specific for IL1-beta has been pre-coated onto the microwells. The IL1-beta protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IL1-beta is added to detect the captured IL1-beta protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm. NB: precursor and active form can be detected by this kit.

## Background

Interleukin-1 is a pro-inflammatory cytokine with multiple biological effects. The IL-1 gene family encodes three proteins: IL-1 $\alpha$ , IL-1 $\beta$  and their naturally occurring inhibitor IL-1RN. Interleukin 1 $\beta$  (IL-1 $\beta$ ), mainly produced by blood monocytes and tissue macrophages, has been implicated in mediating both acute and chronic inflammation. IL-1 $\beta$  is known to be involved in a variety of cellular activities, including cell proliferation, differentiation and apoptosis. IL-1 $\beta$  is emerging as a key mediator of carcinogenesis that characterizes host-environment interactions.

## Sample Preparation

The serum, plasma or cell culture supernatants samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples. The cell lysates is better to be diluted 1:10 before the

assay.

## Safety Notes

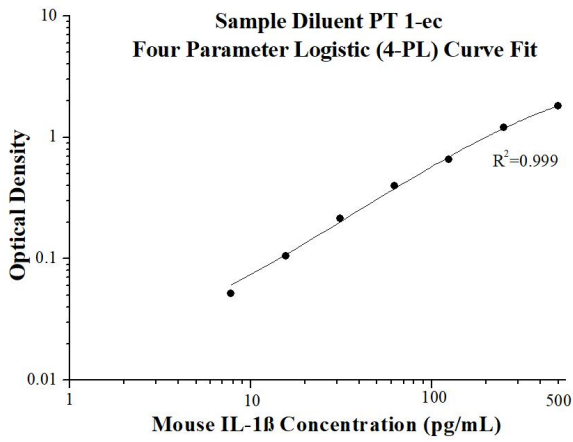
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

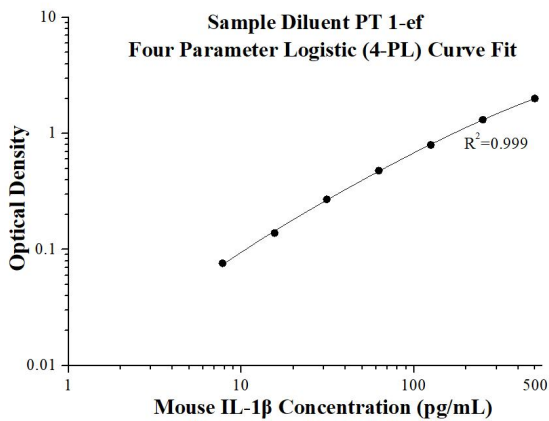
| Step | Reagent  | Volume | Incubation | Wash        | Notes                        |
|------|--|--------|------------|-------------|------------------------------|
| 1    | Standard and Samples   | 100 µL | 120 min    | 4 times     | Cover Wells incubate at 37°C |
| 2    | Diluent Antibody Solution  | 100 µL | 60 min     | 4 times     | Cover Wells incubate at 37°C |
| 3    | Diluent HRP Solution   | 100 µL | 40 min     | 4 times     | Cover Wells incubate at 37°C |
| 4    | TMB Substrate  | 100 µL | 15-20 min  | Do not wash | Incubate in the dark at 37°C |
| 5    | Stop Solution  | 100 µL | 0 min      | Do not wash | -                            |
| 6    | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. |        |            |             |                              |

## Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



| (pg/mL) | O.D            | Average | Corrected |
|---------|----------------|---------|-----------|
| 0       | 0.085<br>0.076 | 0.0805  | -         |
| 7.8     | 0.132<br>0.133 | 0.1325  | 0.052     |
| 15.6    | 0.179<br>0.191 | 0.185   | 0.1045    |
| 31.25   | 0.285<br>0.304 | 0.2945  | 0.214     |
| 62.5    | 0.482<br>0.475 | 0.4785  | 0.398     |
| 125     | 0.773<br>0.696 | 0.7345  | 0.654     |
| 250     | 1.265<br>1.305 | 1.285   | 1.2045    |
| 500     | 1.893<br>1.902 | 1.8975  | 1.817     |



| (pg/mL) | O.D            | Average | Corrected |
|---------|----------------|---------|-----------|
| 0       | 0.048<br>0.05  | 0.049   | -         |
| 7.8     | 0.124<br>0.126 | 0.125   | 0.076     |
| 15.6    | 0.188<br>0.187 | 0.1875  | 0.1385    |
| 31.25   | 0.334<br>0.305 | 0.3195  | 0.2705    |
| 62.5    | 0.531<br>0.524 | 0.5275  | 0.4785    |
| 125     | 0.876<br>0.817 | 0.8465  | 0.7975    |
| 250     | 1.384<br>1.346 | 1.365   | 1.316     |
| 500     | 2.054<br>2.051 | 2.0525  | 2.0035    |

## Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

| Intra-assay Precision |    |              |      |     |
|-----------------------|----|--------------|------|-----|
| Sample                | n  | Mean (pg/mL) | SD   | CV% |
| 1                     | 20 | 520.0        | 17.0 | 3.3 |
| 2                     | 20 | 159.5        | 5.6  | 3.5 |
| 3                     | 20 | 29.1         | 0.5  | 1.8 |

| Inter-assay Precision |    |              |      |     |
|-----------------------|----|--------------|------|-----|
| Sample                | n  | Mean (pg/mL) | SD   | CV% |
| 1                     | 24 | 485.5        | 20.1 | 4.1 |
| 2                     | 24 | 156.6        | 4.8  | 3.1 |
| 3                     | 24 | 28.5         | 1.3  | 4.6 |

## Recovery

The recovery of IL1-beta spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

| Sample Type               |      | Average% of Expected | Range (%) |
|---------------------------|------|----------------------|-----------|
| Mouse serum               | 1:2  | 95                   | 91-99     |
|                           | 1:4  | 109                  | 101-123   |
| Cell culture supernatants | 1:2  | 112                  | 94-123    |
|                           | 1:4  | 114                  | 89-126    |
| Cell lysates              | 1:10 | 107                  | 83-128    |
|                           | 1:20 | 101                  | 82-128    |

## Sample Values

The mouse was injected with LPS (2mg LPS / Kg, 200 ug/mL) and bled after 6 hours. Then the IL1-beta level of mouse serum was tested as 54.2 pg/mL.

**Tissue homogenates** - Dissect the tissue of interest and wash briefly with chilled **1X PBS** to remove any blood if necessary, cut the tissue into smaller pieces whilst keeping it on ice. Transfer the tissue to a homogenizer and add **Extraction Reagent** with protease inhibitor. In general, add 500  $\mu$  L **Extraction Reagent** for approximately every 10 mg of tissue. Homogenize thoroughly and keep the sample on ice for 30 min. Sonicate the sample and centrifuge at 10,000 x g, then transfer the supernatants to assay.

|              | IL1-beta (pg/mL) | Total protein (mg/mL) |
|--------------|------------------|-----------------------|
| Mouse heart  | 854              | 5                     |
| Mouse liver  | 6,908            | 15                    |
| Mouse spleen | 12,018           | 9.2                   |
| Mouse brain  | 2,231            | 17                    |
| Mouse lung   | 3,110            | 9                     |
| Mouse kidney | 10,400           | 18                    |

\***1X PBS** For 1000 mL

10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM NaH<sub>2</sub>PO<sub>4</sub>, 140 mM NaCl. Adjust pH to 7.4 and add ddH<sub>2</sub>O to 1000 mL.

## Sensitivity

The minimum detectable dose of mouse IL1-beta is 1.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of mouse IL1-beta in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The cell lysates sample were initially diluted 1:10)

|      |                      | Mouse serum<br>(Sample Diluent PT 1-ec) | Cell culture supernatants<br>(Sample Diluent PT 1-ef) | Cell lysates<br>(Sample Diluent PT 1-ec) |
|------|----------------------|---|---|--|
| 1:2  | Average% of Expected | 101                                     | 105   | 83                                       |
|      | Range (%)            | 95-108                                  | 92-111  | 79-90                                    |
| 1:4  | Average% of Expected | 103                                     | 108   | 86                                       |
|      | Range (%)            | 101-110                                 | 102-116   | 85-89                                    |
| 1:8  | Average% of Expected | 108                                     | 105   | 88                                       |
|      | Range (%)            | 95-120                                  | 97-110  | 79-105                                   |
| 1:16 | Average% of Expected | 102                                     | 110   | 95                                       |
|      | Range (%)            | 99-111                                  | 97-110  | 91-100                                   |

## References

1. Dinarello CA. et al. (1996). Blood. 87: 2095-147.
2. Bird S. et al. (2002). Cytokine Growth Factor Rev. 13: 483-502.
3. Xu J. et al. (2013). PLoS One. 21;8(5):e63654.
4. McCarty S. (2014). Cardiol Rev. 22: 176-81.