MULTI SCIENCES

# Mouse IL-1β ELISA Kit

【Catalog No】EK201B

【SIZE】48T/96T

【INTENDED USE】For the quantitative determination of mouse Interleukin 1 beta (IL-1β) concentrations in cell culture supernates, serum and plasma.

【INTRODUCTION】

Interleukin-1 (IL-1) is an extracellular peptide of 17 kDa that designates two proteins, IL-1α and IL-1β. IL-1β is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). This cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. The interleukin-1 (IL-1) species represent an important family of biologically active mono nuclear cell-derived proteins which are involved in inflammatory reactions and in immune responses.

Increased production of IL-1β causes a number of different autoinflammatory syndromes, most notably the monogenic conditions referred to as CAPS, due to mutations in the inflammasome receptor NLRP3 which triggers processing of IL-1β. This kit is designed to recognize the cleaved, mature form and uncleaved pro-form of mouse IL-1β.

【PRINCIPLE OF THE ASSAY】

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-1β has been pre-coated onto a microplate. Standard, samples and biotin-linked detect antibody specific for IL-1β are pipetted into the wells and IL-1β present is bound by the immobilized antibody and detect antibody following incubation. After washing away any unbound substances, streptavidin-HRP is added. After washing, substrate solution is added to the wells and color develops in proportion to the amount of IL-1β bound in the initial step. The color development is stopped and the intensity of the color is measured.

【MATERIALS PROVIDED】

| PART | PART # | EK201B-48 | EK201B-96 |
|---|---|---|---|
| Coated Microplate | EK201BP | 48T | 96T |
| Standard | EK201BS | 1 vial | 2 vials |
| Detect antibody | EK201BD | 1 vial | 1 vial |
| Standard Diluent | E0260 | 5 mL | 5 mL |
| Streptavidin-HRP | E0290 | 1 vial | 1 vial |
| Assay Buffer (10×) | E0310 | 5 mL | 5 mL |
| TMB | E0230 | 6 mL | 11 mL |
| Stop Solution | E0300 | 11 mL | 11 mL |
| Washing Buffer (20×) | E0281 | 50 mL | 50 mL |
| Adhesive Film | E0200 | 6 | 6 |

**Note: Components from reagent kits of different batch numbers must not be used interchangeably.**

OTHER SUPPLIES REQUIRED

1) Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.

2) Pipettes and pipette tips.

3) 50 μl to 300 μl adjustable multichannel micropipette with disposable tips.

4) Multichannel micropipette reservoir.

5) Beakers, flasks, cylinders necessary for preparation of reagents.

6) Deionized or distilled water.

7) Polypropylene test tubes for dilution.

【STORAGE】

Store at 2-8℃; refer to the kit label for expiration date.

For opened kits:

Pre-coated microplate: Can be stored at 2-8℃ for approximately 1 month (return unused strips to the aluminum foil bag and reseal).

Standard: Can be stored at -20℃ for approximately 1 month (discard after single-use reconstitution).

Other components: Can be stored at 2-8℃ for approximately 1 month.

【SAMPLE COLLECTION AND STORAGE】

1) **Cell Culture Supernates** - Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

2) **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 × g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

3) **Plasma** - Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000 × g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

4) Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay. Dilution with Assay Buffer may be needed.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

If samples are to be run within 24 hours, they may be stored at 2 to 8℃. For longer storage, aliquot samples and store frozen at -20℃. Avoid repeated freeze-thaw cycles.

【REAGENT PREPARATION】

Bring all reagents and samples to room temperature before use.

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

**Washing Buffer (1×)**

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with pure or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

**Assay Buffer (1×)**

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

**Detect Antibody**

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube.

**The diluted Detect Antibody should be used within 30 minutes after dilution.**

**Streptavidin-HRP**

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed.

**The diluted Streptavidin-HRP should be used within 30 minutes after**

**dilution.**

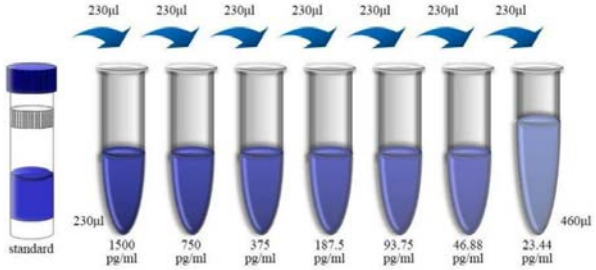
**Sample Dilution:** If your samples have high IL-1β content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

**Mouse IL-1β Standard:** Briefly spin the vial at 6,000 rpm for 30 seconds before opening. Reconstitute mouse IL-1β Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 3,000 pg/ml).

Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

Use polypropylene tubes.

- **For serum/plasma samples,** mixing concentrated mouse IL-1β standard (230 µl) with 230µl of Standard Diluent creates the high standard (1,500 pg/ml). Pipette 230 µl of Standard Diluent into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. Standard Diluent serves as the zero standard (0 pg/ml).
- **For cell culture supernates,** mixing concentrated mouse IL-1β standard (230 µl) with 230 µl of cell culture medium creates the high standard (1,500 pg/ml). Pipette 230 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).




**【ASSAY PROCEDURE】**

Bring all reagents and samples to room temperature before use.

1) Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.

2) Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.

3) Add 300 µl Washing Buffer (1×) per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer (1×). Use the microwell strips immediately after washing. **Do not allow wells to dry.**

4) Add 100 µl 2-fold diluted Standard to Standard well. Add 100 µl Standard Diluent/ culture medium to Blank well.

5) **Serum/Plasma**: Add 90 µl Assay Buffer (1×) and 10 µl sample to the sample well. **Cell culture supernates**: Add 100 µl cell culture supernates to the sample well.

6) Add 50 µl of diluted Detect Antibody to each well. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.

7) **Seal the plate with an adhesive film.** Incubate at room temperature (25℃±3℃) for 2 hours on a microplate shaker set at 300 rpm.

8) Aspirate each well and wash by filling each well with 300 µl Washing Buffer (1×), repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining Washing Buffer (1×) by aspirating or decanting. Invert the plate and tap it against clean paper towels.

9) Add 100 µl of diluted Streptavidin-HRP to each well.

10) **Seal the plate with a fresh adhesive film.** Incubate at room temperature (25℃±3℃) for 45 minutes on a microplate shaker set at 300 rpm.

11) Repeat aspiration/wash as in step 8.

12) Add 100 µl of Substrate Solution to each well. Incubate for 5 - 30 minutes at room temperature(25℃±3℃). **Protect from light.**

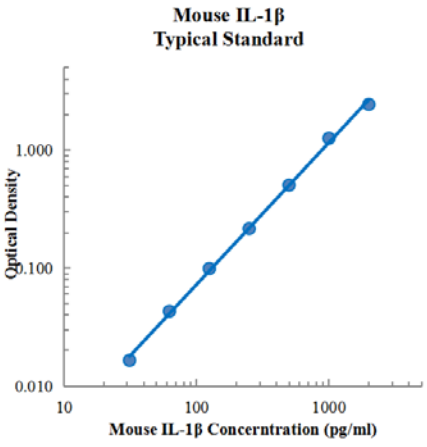
13) Add 100 µl of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

14) Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

**【TYPICAL DATA】**

A standard curve must be run within each assay. The following standard curve is provided for demonstration only.

| pg/ mL | O.D. | | Average | Corrected |
|---|---|---|---|---|
| 0.00 | 0.009 | 0.01 | 0.009 | |
| 23.44 | 0.026 | 0.026 | 0.026 | 0.017 |
| 46.88 | 0.053 | 0.052 | 0.053 | 0.043 |
| 93.75 | 0.109 | 0.108 | 0.109 | 0.099 |
| 187.50 | 0.233 | 0.218 | 0.226 | 0.216 |
| 375.00 | 0.515 | 0.514 | 0.515 | 0.505 |
| 750.00 | 1.303 | 1.237 | 1.270 | 1.261 |
| 1500.00 | 2.456 | 2.439 | 2.448 | 2.438 |




**MULTI SCIENCES BIOTECH CO., LTD.**
**www.multisciences.net**
**E-mail: service@multisciences.net**