MULTI SCIENCES

EasyGo!™ Rat IL-1β One-Step ELISA Kit

【Catalog No】EK301BEGB

【SIZE】48T/96T

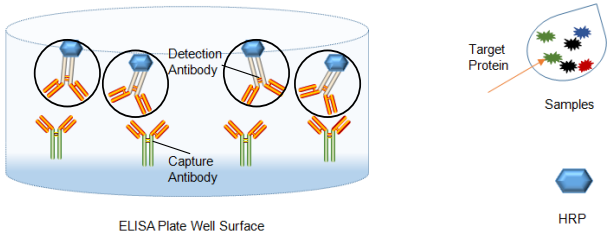
【INTENDED USE】For the quantitative determination of rat Interleukin 1β (IL-1β) concentrations in cell culture supernates.

【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.

【PRINCIPLE OF THE ASSAY】



This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Rat IL-1β has been immobilized onto microwells, and two pellets of the biotin-linked detect antibody specific for IL-1β (light yellow) and streptavidin-HRP (purple) are pre-placed in the microwells, sealed by the adhesive film. Standard or samples are pipetted into the wells, then IL-1β present is bound by the immobilized antibody and detect antibody, of which the latter is conjugated with streptavidin-HRP in the incubation. After washing, substrate solution reacts with HRP and color develops in proportion to the amount of IL-1β bound by the immobilized antibody. The color development is stopped and the intensity of the color is measured by microplate reader.

【MATERIALS PROVIDED】

PART	PART #	EK301BEGB-48	EK301BEGB-96
Coated Microplate	EK301BEGBP	48T	96T
Standard	EK301BEGBS	1vial	2vials
TMB	E0230	6mL	11mL
Stop Solution	E0300	11mL	11mL
Washing Buffer (20×)	E0281	11mL	11mL

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) Other biological samples might be suitable for use in the assay. Cell culture supernates 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.

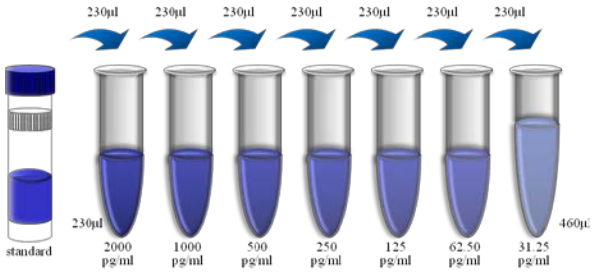
Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Sample Dilution: If your samples have high **IL-1β** content, dilute cell culture supernates with cell culture medium.

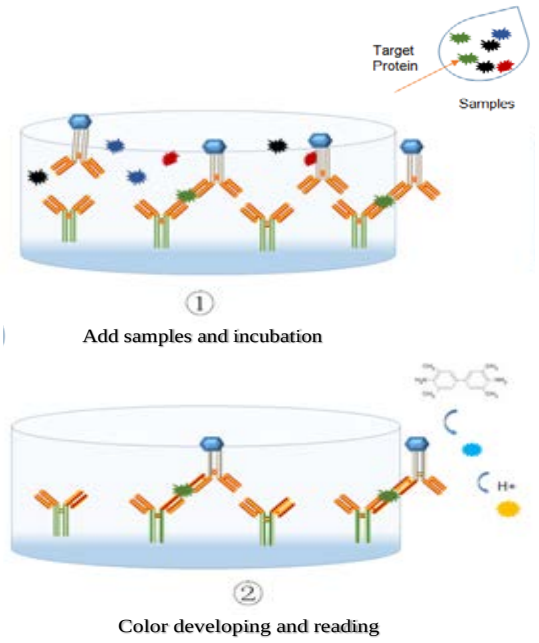
Rat IL-1β Standard: Reconstitute **Rat IL-1β Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 4,000 pg/ml).

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

- **For cell culture supernates,** mixing concentrated Rat IL-1β standard (230 μl) with 230 μl of cell culture medium creates the high standard (2,000 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 with the knife and return them to the foil pouch containing the desiccant pack, and reseal for further use.**In any case, avoid touching the inner surface of the microwells and gently tap the plate to ensure that the pellets on the bottom of the microwells. Do not discard the pellets.**

3) Add 100 µl 2-fold diluted Standard to Standard well. Add 100 µl culture medium to Blank well.**The standards/samples can be added directly from the middle opening of the plate adhesive film.**

4) **Cell culture supernates**: Add 100 µl cell culture supernates to the sample well.

5) Incubate at 37℃ for **1** hour, or at room temperature (25℃±3℃) for **2** hours, on a microplate shaker set at 300-500 rpm.

6) Removing the plate adhesive film. Aspirate each well and wash by filling each well with 300 µl Washing Buffer (1×), repeat three times for a total four washes with 60 seconds interval. 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.

7) Add 100 µl of Substrate Solution to each well. Incubate for 10±5 minutes at room temperature(25℃±3℃). **Protect from light.**

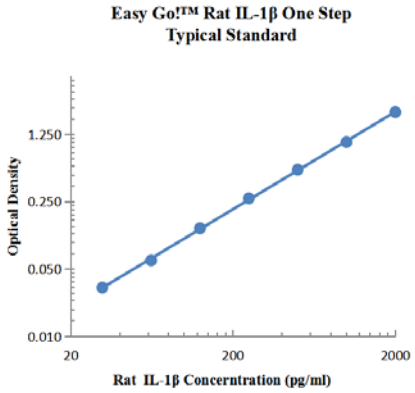
8) Add 100 µl of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9) 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	0.017	0.020	0.019	
31.25	0.049	0.052	0.051	0.032
62.50	0.082	0.078	0.080	0.062
125.00	0.150	0.152	0.151	0.133
250.00	0.279	0.299	0.289	0.271
500.00	0.552	0.560	0.556	0.538
1000.00	1.059	1.061	1.060	1.042
2000.00	2.192	2.097	2.145	2.126



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