



Human IL-1 β ELISA

Catalog Number EA-0531

(For Research Use Only)

Introduction

IL-1 β is a member of the interleukin-1 family of cytokines. It non-productively binds the IL-1 β receptor thereby modulating the pro-inflammatory effects of IL-1 α and IL-1 β . Mutations in IL-1 β have been linked to osteoporotic fractures, gastric cancer, schizophrenia, and the rare disease, Deficiency of the Interleukin-1-Receptor Antagonist (DIRA). Recombinant IL-1 β is currently used to treat autoimmune disorders and lymphomas.

Principle of the assay

IL-1 β ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes an anti-human IL-1 β antibody for immobilization on the microtiter wells and a biotinylated anti-human IL-1 β antibody along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the IL-1 β molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added, which results in the development of a blue color. The color development is then stopped with the addition of stop solution changing the color to yellow. The concentration of IL-1 β is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

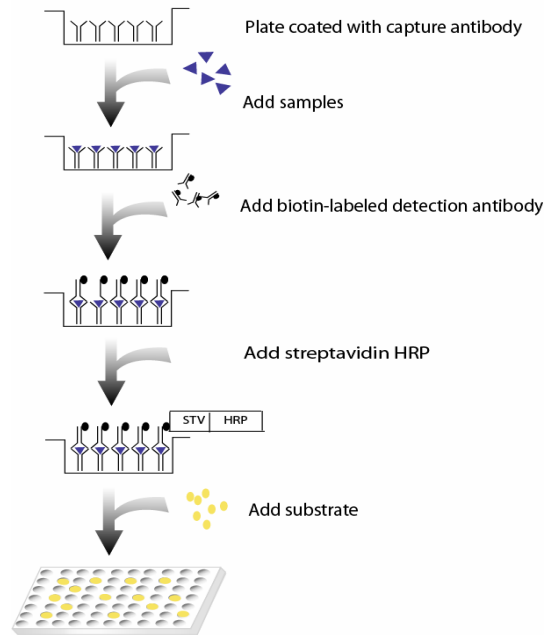


Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
8x12 96-well microplate coated with anti-human IL-1 β antibodies	1	4°C
Biotin-labeled goat anti-human IL-1b antibodies	25 μ L	-20°C
Recombinant Human IL-1 β standard (400ng/ml)	10 μ L	-20°C
Streptavidin-HRP conjugate	50 μ L	4°C
1X Diluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	4°C

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5X Assay wash buffer to 1X buffer
40ml 5X Assay wash buffer
160ml ddH₂O
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- **Standard Curve Preparation:** Prepare 500 pg/ml Human IL-1 β standard by diluting 1 μ l of the provided Human IL-1 β standard (400 ng/ml) in 400 μ l 1X Diluent Buffer. Then, do 4-fold serial dilutions six times (Standard curve is 7 wells plus 1 blank well). Add 100 μ l of the diluted standards to each well.

Standard#	IL-1 β Concentration (pg/ml)
1	500
2	125
3	31.25
4	7.81
5	1.95
6	0.488
7	0.122
8	0

- Dilute biotin-labeled anti-human IL-1 β 1:400 with 1X Diluent buffer before use.
- Dilute streptavidin-HRP 1:200 with 1X Diluent buffer before use.
- Avoid contact of Substrate and Stop Solution with any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50 μ L of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Add 100 μ l of sample or standard per well and incubate for 1-2 hours at room temperature (or overnight at 4°C) with gentle shaking.
3. Aspirate each well and wash by adding 200 μ l of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100 μ l of diluted biotin-labeled anti-human IL-1 β antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 μ l of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
9. Add 100 μ l of substrate to each well and incubate for 10-30 minutes.
10. Add 50 μ l of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Immediately measure the plate with a microplate reader at 450 nm.