



AssayMax Human Interleukin-1beta (IL-1beta) ELISA Kit

Catalog # EI2200-1

Introduction

Interleukin-1beta (IL-1beta) has a wide spectrum of inflammatory, metabolic, haemopoietic, and immunological properties (1). IL-1 beta plays a significant role in hippocampal synaptic function (2), and is a potential genetic marker as indicator of gastric cancer risk (3).

High plasma level of Interleukin-1 beta (IL-1b) is associated with rheumatoid and osteoarthritic joint disease (4), infectious gastroenteritis (5), neurodegeneration (6), and breast cancer (7). High gingival crevicular fluid levels of interleukin-1beta is related to type 2 diabetes (8).

Principal of the Assay

The AssayMax Human IL-1beta ELISA kit is designed for detection of human IL-1beta in plasma, serum, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique which measures IL-1beta in 5 hours. A murine monoclonal antibody specific for IL-1beta has been pre-coated onto a microplate. IL-1beta in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for IL-1beta, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **IL-1beta Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against IL-1beta.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.

- **IL-1beta Standard:** Human IL-1beta in a buffered protein base (0.5 ng, lyophilized).
- **Biotinylated IL-1beta Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human IL-1beta (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (120 µl)
- **EIA Diluent Concentrate (10x):** A 10-fold buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x):** A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8⁰C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8⁰C. Store reconstituted standard at -20⁰C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20⁰C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **Standard Curve:** Reconstitute the 0.5 ng of human IL-1beta Standard with 2 ml of EIA Diluent to generate a stock solution of 250 pg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the IL-1beta standard solution (250 pg/ml) twofold with equal volume of EIA

Diluent to produce 125.0, 62.5, 31.3, 15.7, 7.8, and 3.9 pg/ml solutions. EIA Diluent serves as the zero standard (0 pg/ml).

Standard Point	Dilution	[IL-1beta] (pg/ml)
P1	1 part Standard (250 pg/ml)	250.0
P2	1 part P1 + 1 part EIA Diluent	125.0
P3	1 part P2 + 1 part EIA Diluent	62.5
P4	1 part P3 + 1 part EIA Diluent	31.3
P5	1 part P4 + 1 part EIA Diluent	15.7
P6	1 part P5 + 1 part EIA Diluent	7.8
P7	1 part P6 + 1 part EIA Diluent	3.9
P8	EIA Diluent	0.0

- **EIA Diluent Concentrate (10x):** Dilute EIA Diluent Conc. 1:10 with reagent grade water.
- **Biotinylated IL-1beta Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent.
- **Wash Buffer Concentrate (10x):** Dilute Wash Buffer Conc. 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

Assay Procedure

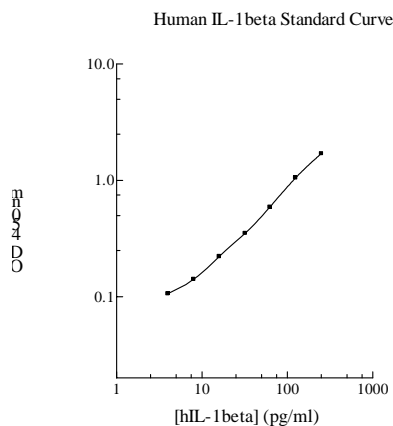
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- Add 50 µl of Biotinylated IL-1beta Antibody to each well and incubate for two hours.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using 4-parameter or log-log curve fit.
- Determine the unknown sample concentration from the Standard Curve.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable level of IL-1beta is typically < 3 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1 % and 7.5% respectively.
- No significant cross-reactivity or interference was observed.

References

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