AssayMax Mouse Interleukin-1β (IL-1 β) ELISA Kit

Catalog # EMI2200-1

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 Mouse IL-1beta ELISA kit is designed for detection of Mouse 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 mouse 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:** Mouse IL-1beta in a buffered protein base (2 ng, lyophilized).
- **Biotinylated IL-1beta Antibody (100x):** A 100-fold biotinylated polyclonal antibody against Mouse IL-1beta (80 μl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 μl).

- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}C 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, 200-1000 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 -20^{0}C 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 -20^{0}C 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 -20^{0}C 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.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **Standard Curve:** Reconstitute the 2 ng of Mouse IL-1beta Standard with 2 ml of MIX Diluent to generate a standard solution of 1 ng/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 (1 ng/ml) twofold with equal volume of MIX Diluent to produce 0.5, 0.25, 0.125, 0.063, 0.031, and 0.016 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[IL-1beta] (ng/ml)
P1	1 part Standard (1 ng/ml)	1.000
P2	1 part P1 + 1 part MIX Diluent	0.500
P3	1 part P2 + 1 part MIX Diluent	0.250
P4	1 part P3 + 1 part MIX Diluent	0.125
P5	1 part P4 + 1 part MIX Diluent	0.063
P6	1 part P5 + 1 part MIX Diluent	0.031
P7	1 part P6 + 1 part MIX Diluent	0.016
P8	MIX Diluent	0.000

- **Biotinylated IL-1beta Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** Dilute Wash Buffer 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20^{0}C.

Assay Procedure

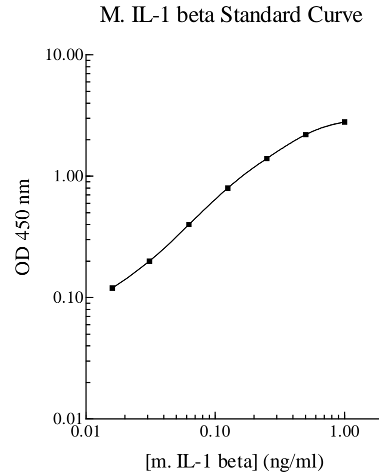
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. 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 with a sealing tape 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 complete 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 8 minutes or until 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 log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

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 < 10 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.1 % and 7.0% respectively.

Cross-Reactivity

- No significant cross-reactivity or interference was observed.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Cell Culture Supernatant**
1:10	105%
1:20	100%
1:40	100%

Recovery

Standard Added Value	0.1 – 1 ng/ml
Recovery %	88-112 %
Average Recovery %	100 %

References

(1) Palmi M *et al*. (2002) *Mol Neurobiol.* 25(2): 133-47
(2) Lynch MA (2002) *Vitam Horm.* 64:185-219
(3) Troost E *et al.* (2003) *Can J Gastroenterol.* 17 Suppl B: 8B-12B
(4) Fan Z *et al.* (2004) *Cytokine* 28(1): 17-24
(5) Enocksson A *et al.* (2004) *Clin Diagn Lab Immunol.* 11(2): 250-4
(6) Allan SM *et al.* (2005) *Nat Rev Immunol* 5(8): 629-40
(7) Mettler L *et al.* (2004) *Clin Exp Obstet Gynecol.* 31(1): 20-2
(8) Engebretson SP *et al.* (2004) *J Periodontol.* 75(9): 1203-8

Version 1.2