Human Interleukin-1α (IL-1α) ELISA Kit

Introduction

Interleukin-1 alpha (IL-lα) is a member of the IL-1 superfamily containing IL-lα, IL-lβ, and IL-1Ra receptor antagonist. IL-lα is known as hematopoietin (IL-1F1) and IL-lβ as catabolin (IL-1F2). IL-lα and IL-lβ are corresponding to two different isoelectric forms, acidic pI 5 and neutral pI 7, respectivelybeta. IL-lα has a molecular mass of 17 kDa and consist of 159 amino acids having 26-33% homology with IL-lβ (1-6). They are produced mainly by macrophages and monocytes, processed and released during apoptosis, and bind with high affinity to specific receptors on target cells. While only the mature form of IL-lβ has biological activity, both the pro and mature forms of IL-lα are active (7). IL-lα and -β are pro-inflammatory cytokine involved in immune responses, inflammatory reactions, and hematopoiesis (8-9). IL-lα is an epidemal cytokine that is constitutively produced by epithelial cells and plays important role in maintenance of skin barrier function (10). The polymorphism of increased IL-1 production in patients is associated with rheumatoid arthritis, coagulation, solid tumors, leukemias, Alzheimer’s disease, autoimmune disorders, and myocardial infarction (11).

Principal of the Assay

The Human IL-1α ELISA kit is designed for detection of human IL-1α in plasma, serum, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique, which measures IL-1α in 5 hours. A murine monoclonal antibody specific for IL-1α has been pre-coated onto a microplate. IL-1α in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for IL-1α, 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-1α Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against IL-1α.

- **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-1α Standard:** Human IL-1α in a buffered protein base (125 pg, lyophilized).
- **Biotinylated IL-1 α Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human IL-1α (80 μl).
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 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 kit at 2-8^0C or -20^0C upon arrival up to the expiration date.
- Opened EIA Diluent may be stored for up to 1 month at 2-8^0C. Store reconstituted reagents at -20^0C 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 -20^0C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **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 -20^0C 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 -20^0C 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.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^0C.
- **Standard Curve:** Reconstitute the 125 pg of human IL-1α Standard with 0.5 ml of EIA Diluent to generate a standard solution of 250 pg/ml. Allow the standard to sit for 10

minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the IL-1α standard solution (250 pg/ml) 1:4 with EIA Diluent to produce 62.5, 15.7, 3.91, and 0.98 pg/ml solutions. EIA Diluent serves as the zero standard (0 pg/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[IL-1α] (pg/ml)
P1	1 part Standard (250 pg/ml)	250.0
P2	1 part P1 + 3 parts EIA Diluent	62.5
P3	1 part P2 + 3 parts EIA Diluent	15.7
P4	1 part P3 + 3 parts EIA Diluent	3.91
P5	1 part P4 + 3 parts EIA Diluent	0.98
P6	EIA Diluent	0.00

- **Biotinylated IL-1α Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA 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 EIA Diluent. Any remaining solution should be frozen at -20^{0}C.

Assay Procedure

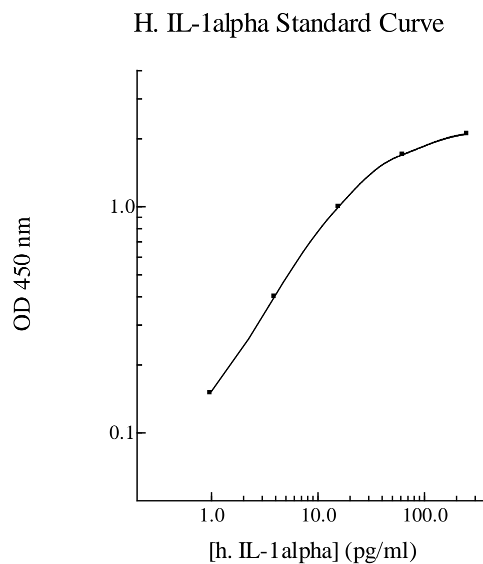
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. 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 manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated IL-1α Antibody to each well and incubate for two hours.
- Wash a microplate as described 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 a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 20 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**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. 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 duplicate or 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-1α is typically < 3 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.5 % and 7.1% respectively.

Cross-Reactivity

- No significant cross-reactivity or interference was observed.

References

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