Human IL-1β ELISA Kit

Catalog No. BSKH1001 (96 wells)

For Use with serum, plasma and cell culture supernatants

For Research Use Only. Not for use in diagnostic procedures.

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Introductions

Interleukin-1 (IL-1), originally described as lymphocyte activating factor (LAF) for its effects on thymocytes, is a polypeptide cytokine with two molecular forms. The two distinct molecular forms of IL-1 are thought to be derived from two genes. After transcription, as 31kD precursor polypeptide is cleaved to give rise to mostly cell membrane associated IL-1α and secreted IL-1β. Both have the same molecular weight of 15kD but have different isoelectric points of 5 and 7, respectively.

Despite sequence homology of only 20%, both forms are thought to bind to the same receptor. IL-1 inhibitors that vary only in their degree of glycosylation have been described to bind to the IL-1 receptor. These inhibitors are structurally related to IL-1β and may be important in regulation of IL-1β action.

IL-1β is produced primarily by monocytes and macrophages but also by astrocytes, oligodendroglia, adrenal cortical cells, NK cells, endothelial cells, keratinocytes, megakaryocytes, platelets, neurons, neutrophils, osteoblasts, Schwann cells, trophoblasts, T cells, and fibroblasts. IL-1 has multiple immunological functions including enhancement of IL-2 production by T cells and activation of B-cells (BAF) and thymocytes. A true pleiotrope, IL-1 may have tumoricidal activity via its release of IL-2 and Interferon gamma and indirectly antiviral by stimulating to release interferon beta.

Low levels of IL-1β have been reported in normal serum. It is thought that IL-1 genes are induced to respond to tissue damage or infection. Elevated levels have been reported in a number of infectious disease conditions and in noninfectious inflammatory conditions such as Crohn's disease. In addition to elevated serum levels, IL-1 has been found in synovial fluids of patients with rheumatoid arthritis and in cerebrospinal fluid after neurological inflammation or insult. At the other end of the spectrum, low levels of IL-1 have been found in malnutrition and advanced neoplasia suggesting perhaps a complex immunological and physiological regulatory role for this cytokine.

Principle of the Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1β present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for IL-1β is added to the wells and binds to the combination of capture antibody- IL-1β in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of IL-1β present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven IL-1β standard dilutions and IL-1β sample concentration determined.

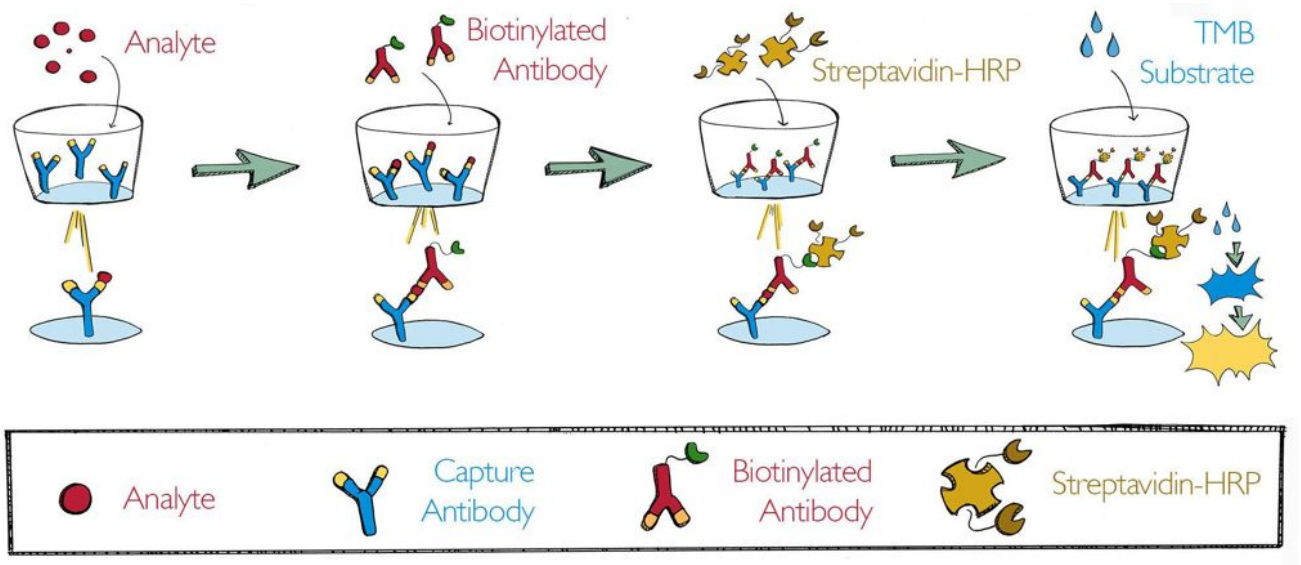


Figure 1. Schematic diagram of the assay

Materials supplied

Table 1. Kit Components

Kit Components	96 wells Quantity/Size
Aluminium pouches with a Microwell Plate coated with monoclonal antibody to human IL-1β (8-12)	1 plate
Human IL-1β Standard lyophilized, 500 pg/ml upon reconstitution	2 vials
Concentrated Biotin-Conjugate anti-human IL-1β monoclonal antibody	2 vials
Streptavidin-HRP solution	2 vials
Standard /sample Diluent	1 bottle
Biotin-Conjugate antibody Diluent	1 bottle
Streptavidin-HRP Diluent	1 bottle
Wash Buffer Concentrate 20x (PBS with 1% Tween-20)	1 bottle
Substrate Solution	1 vial
Stop Solution	1 vial
Adhesive Films	4 pieces
Product data sheet	1 copy

Storage

Table 2. Storage of the kit

Unopened Kit	Shipped at room temperature. Store at 2 - 8°C. Do **NOT** use past kit expiration date**!**	
Opened/ Reconstituted Reagents	Standard /Sample Diluent	May be stored for up to 1 month at 2 - 8°C.**
	Concentrated Biotin-Conjugate	
	Streptavidin-HRP Solution	
	Biotin-Conjugate Antibody Diluent	
	Streptavidin-HRP Diluent	
	Wash Buffer Concentrate 20x	
	Substrate Solution	
	Stop Solution	
	Standard	Aliquot and store for up to 1 month at ≤20°C. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.**

**Provided this is within the expiration date of the kit.

Materials Needed but Not Supplied

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000ul.
3. 37℃ incubator.
4. Double-distilled water or deionized water.
5. Coordinate paper.
6. Graduated cylinder.

Precautions for Use

1. Store kit reagents between 2℃and 8℃.
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or

specimens. In the case of contact with skin or eyes wash immediately with water.

5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on the label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

Sample Collection and Storage

1. **Cell Culture Supernatants** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor). **Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.**

Reagent Preparation

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 1.0mL of Standard /sample Diluent. This reconstitution produces a stock solution of 500 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 500uL of Standard/sample Diluent into the 250 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 500 pg/mL standard serves as the high standard. The Standard/ sample Diluent

serves as the zero standard (0 pg/mL).

If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

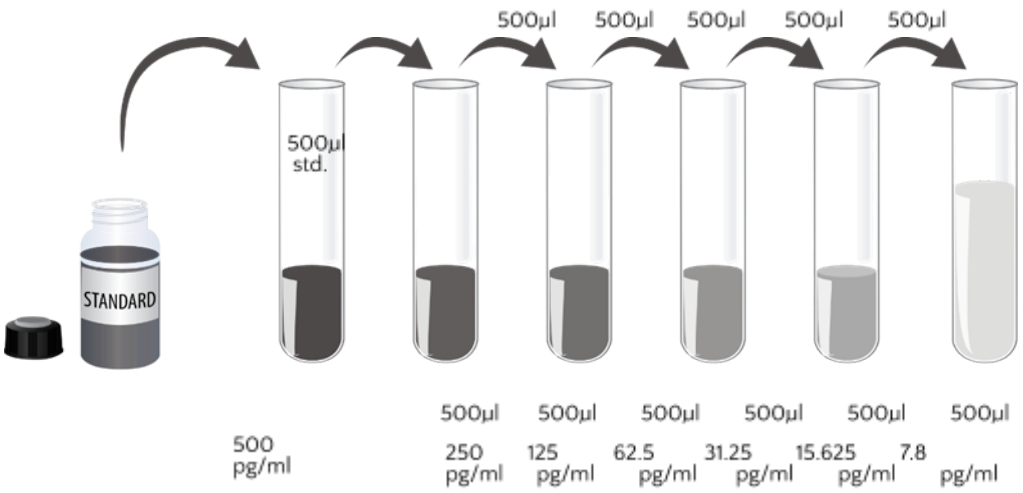


Figure 2. Preparation of IL-1β standard dilutions

4. Working solution of Biotin-Conjugate anti-human IL-1β monoclonal antibody:

   Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

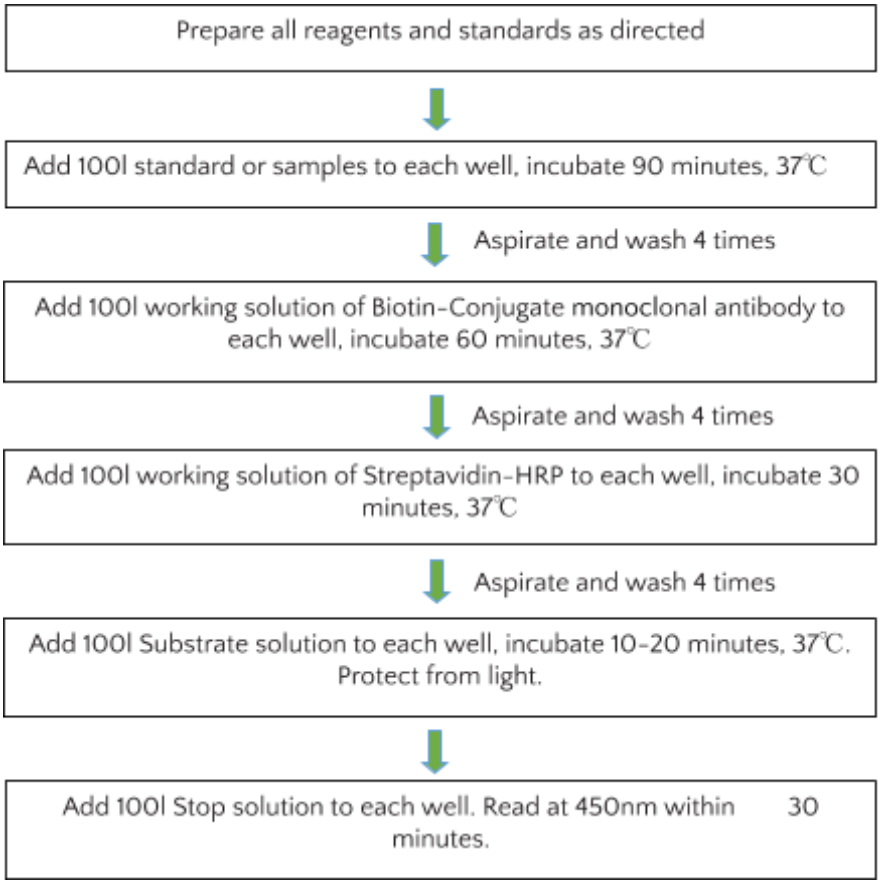
   The working solution should be used within one day after dilution.

General ELISA Protocol

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃, sealed tightly.
3. Add 100µL of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37℃.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µL) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hours at 37℃.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37℃. Avoid placing the plate in direct light.

8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 10-20 minutes at 37℃. Avoid placing the plate in direct light.
10. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. (optionally 630nm as the reference wavelength; 610-650nm is acceptable)

Assay Procedure Summary



Technical Hints

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

Calculation of Results

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. The data may be linearized by plotting the log of the IL-1β concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. This standard curve below is provided ***for demonstration only***. A standard curve should be generated for each set of samples assayed.

Table 3. Typical data using the IL-1β ELISA (Measuring wavelength: 450nm, Reference wavelength: 630nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.045	0.046	0.046	——
7.8	0.143	0.139	0.141	0.095
15.625	0.218	0.216	0.217	0.171
31.25	0.394	0.389	0.392	0.346
62.5	0.715	0.716	0.716	0.670
125	1.240	1.238	1.239	1.193
250	2.038	2.032	2.035	1.989
500	2.535	2.540	2.538	2.492

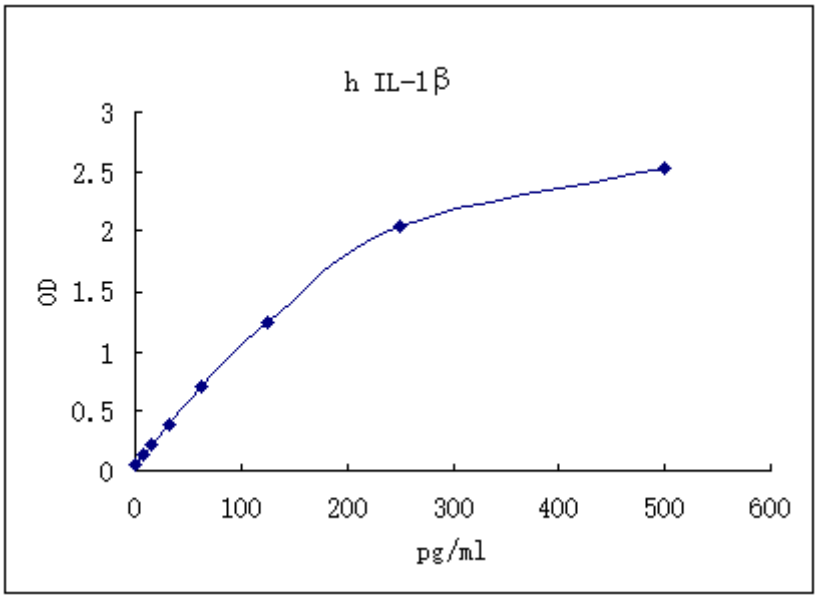


Figure 4. Representative standard curve for human IL-1β ELISA.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Performance Characteristics

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

SENSITIVITY: The minimum detectable dose was 4pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human IL-1β . The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 4. Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
IL-1 α	IL-1 α	IL-1 β
IL-1ra		
IL-1 sRI		
IL-1 sRII		