HUMAN INTERLEUKIN-1 BETA (IL-1 BETA) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF IL-1 BETA CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	IL-1 BETA (HUMAN) ELISA KIT
Catalog No.	SK00746-06
Lot No.	
Formulation	96 T
Standard range	1.95 – 125 pg/mL
Sensitivity	0.5 pg/mL
Sample Volume	100 µL per well
Sample Dilution	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, EDTA Plasma
Specificity	Human IL-1 beta only
Calibration	Human IL-1 beta recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 4 months. See page 2 for detail
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human IL-1 beta ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural IL-1 BETA from plasma and serum samples in a sandwich ELISA format.

This immunoassay contains recombinant IL-1 BETA and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural IL-1 BETA samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for IL-6. The capture antibody can bind to the IL-1 BETA in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against IL-1 BETA is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of IL-1 BETA bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
IL-1 BETA Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human IL-1 beta.	**746-06-01**	**1 plate**
IL-1 BETA Standard – 5000 pg/vial of recombinant IL-1 BETA in a buffered protein base with preservatives; lyophilized.	**746-06-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial of 10-fold concentrate of biotinylated antibody against human IL-1 BETA in liquid form.	**746-06-03**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial of 100-fold concentrated solution of streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB310**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08C**	**1 bottle**
Wash Buffer 20X - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution -11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 4 months. For longer storage for up to 10 months, unopened Standard, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB

Substrate Solution should be stored only at 2 ~ 8 °C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Human plasma and serum samples DO NOT require any dilutions.

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 ml of Wash Buffer Concentrate 20X into deionized or distilled water (475 ml) to prepare 500 ml of Wash Buffer.

IL-1 BETA Standard - Reconstitute the IL-1 BETA standard with 1.0 mL of Dilution Buffer (DB310). This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipet 780 µl of Dilution Buffer into tube #1 and transfer 20 µl of the stock solution (5000 pg/ml) to make the high standard of 125 pg/ml. Pipet 250 µl of Dilution Buffer into tubes #2 to #6 and use the high standard to produce a dilution series (see below). Mix each tube thoroughly before the next transfer (2-fold). The **125 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/ml).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1.0 ml	5000 pg/ml
# 1	20 µl of stock	780 µl	125 pg/ml
# 2	250 µl of 1	250 µl	62.5 pg/ml
# 3	250 µl of 2	250 µl	31.25 pg/ml
# 4	250 µl of 3	250 µl	15.6 pg/ml
# 5	250 µl of 4	250 µl	7.8 pg/ml
# 6	250 µl of 5	250 µl	3.9 pg/ml
# 7	250 µl of 6	250 µl	1.95 pg/ml

Detection Antibody Concentrate - Pipette 9.45 mL of Dilution Buffer (DB310) into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of HRP Diluent Solution (DB08C) into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light). DO NOT FREEZE. The working solution of Streptavidin-HRP Conjugate**

should be freshly prepared and used within in a few hours.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add **100 µL** of Dilution Buffer to Blank wells.
3. Add **100 µL** of standard dilutions in reverse order of serial dilution, samples per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker (350-400 rpm) at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1x Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add **100 µL** of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add **100 µL** of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 2 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

IL-1 BETA (PG/ML)	CORRECTED O.D. (450NM)
Blank	0 (0.099)
1.95	0.029
3.9	0.061
7.8	0.115
15.6	0.239
31.25	0.569
62.5	0.999
125	2.019

- **Lot No.:**

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human IL-1 beta	100
Human IL-1 alpha	0
Human IL-4	0
Human IL-8	0
Human IL-10	0
Human IL-12	0
Human IL-1β	0
Mouse IL-1 beta	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add **100 µl** of standard dilutions, samples to the well. Incubate 2 hours on the plate shaker (250-300 rpm) at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light**.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate solution to each well. Incubate 20 min on the plate shaker at RT. **Protect from light**.
⬇
Add 100 µl Stop Solution to each well. Read 450 nm within 5 min.