Human IL-1β ELISA Kit

Instructions for use

Catalogue numbers:
1x48 tests: EA101142
1x96 tests: EA101143
2x96 tests: EA101144

For research use only

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Human IL-1β ELISA KIT

1. Intended use

The OriGene IL-1β ELISA kit is a solid phase sandwich ELISA for the *in-vitro* qualitative and quantitative determination of IL-1β in supernatants, buffered solutions or serum and plasma samples. This assay will recognise both natural and recombinant human IL-1β.

This kit has been configured for research only .in the rest of the world.

2. Introduction

2.1. Summary

Interleukin-1 Beta (IL-1β) is a member of the interleukin-1 family. This family consists of three structures related polypeptides. The first two are IL-1α and IL-1β, each of which has a broad spectrum of both beneficial and harmful biologic actions, and the third is IL-1-receptor antagonist, which inhibits the activities of interleukin-1.
IL-1 α and β present approximately 25% homology at the amino acid level, but the difference is in their tri dimensional structure. Two distinct receptor types have been isolated, that bind both forms.IL-1β are synthesized as a larger precursor, with a molecular weight 31kda. The molecular weight of the mature form is 17.5kDa.Unlike IL1α, the IL-1β precursor show a little or no biological activity in comparison to the mature form.
IL-1 is primarily an inflammatory cytokine. It belongs to a groups of cytokines with overlapping biologic properties (TNFα and IL-6). IL-1, TNF and IL-6 share the ability to stimulate T and B lymphocytes, increase cell proliferation, and initiate or suppress gene expression for several proteins. It exerts their effects by binding to specific receptors.
IL-1 (α and β) have similar biological properties, among them, the ability to induce fever, sleep, anorexia and hypotension. IL-1 stimulates the release of pituitary hormones, increases the synthesis of collagenases, resulting in the destruction of cartilage, and stimulates the production of prostaglandins, leading to decrease in the pain threshold. In addition IL-1 has some host-defense properties. However, whereas IL-1β is a secreted cytokine, IL-1α is predominantly a cell-associated cytokine.
IL-1 has also been implicated in the destruction of beta cells of the islets of Langerhans, the growth of myelogenous leukaemia cells, and the development of atherosclerotic plaques. It is described in several diseases : sepsis syndrome, rheumatoid arthritis, inflammatory Bowel disease, acute and chronic myelogenous leukaemia, insulino-dependant diabetes mellitus, artherosclerosis.

2.2. Principle of the method

A capture Antibody highly specific for IL-1βhas been coated to the wells of the microtitre strip plate provided during manufacture. Binding of IL-1βsamples and known standards to the capture antibodies and subsequent binding of the biotinylated anti- IL-1βsecondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is removed. The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue coloured complex with the conjugate. The colour development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced coloured complex is directly proportional to the concentration of IL-1βpresent in the samples and standards. The absorbance of the colour complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of IL-1βin any sample tested.

3. Reagents provided and reconstitution

Reagents (Store@2-8^{o}C)	**Quantity 1x48 well kit** Cat no. EA101142	**Quantity 1x96 well kit** Cat no. EA101143	**Quantity 2x96 well kit** Cat no. EA101144	**Reconstitution**
96 well microtitre strip plate	1/2	1	2	Ready to use (Pre-coated)
Plastic plate covers	2	2	4	n/a
Standard: 500 pg/ml	1	2	4	Reconstitute as directed on the vial (see reagent preparation, section 8)
Standard Diluent (Buffer)	1 (25ml)	1 (25ml)	1 (25ml)	10x Concentrate, dilute in distilled water (see reagent preparation, section 8)
Standard Diluent Serum	1 (7ml)	1 (7ml)	2 (7ml)	Ready to use
Control	1	2	4	Reconstitute as directed on the vial (see reagent preparation, section 8)
Biotinylated anti-IL-1b	1 (0.4ml)	1 (0.4ml)	2 (0.4ml)	Dilute in biotinylated antibody diluent (see reagent preparation, section 8)
Biotinylated Antibody diluent	1 (7ml)	1 (7ml)	1 (13ml)	Ready to use
Streptavidin-HRP	1 (5µl)	2 (5µl)	4 (5µl)	Add 0.5ml of HRP diluent prior to use (see reagent preparation, section 8)
HRP Diluent	1 (23ml)	1 (23ml)	1 (23ml)	Ready to use
Wash Buffer	1 (10ml)	1 (10ml)	2 (10ml)	200x Concentrate dilute in distilled water (see reagent preparation, section 8)
TMB Substrate	1 (11ml)	1 (11ml)	1 (24ml)	Ready to use
H_2SO_4 stop reagent	1 (11ml)	1 (11ml)	2 (11ml)	Ready to use

4. Materials required but not provided

- Microtitre plate reader fitted with appropriate filters (450nm required with optional 620nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000µl adjustable single channel micropipettes with disposable tips
- 50-300µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile

5. Storage Instructions

Store kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C). Expiry of the kit and reagents is stated on box front labels. The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6. Specimen collection, processing & storage

Cell culture supernatants, human serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Cell culture supernatants: Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.

Serum: Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

Plasma: EDTA, citrate and heparin plasma can be assayed. Spin samples at 1000 x g for 30 min to remove particulates. Harvest plasma.

Storage: If not analyzed shortly after collection, samples should be aliquoted (250-500µl) to avoid repeated freeze-thaw cycles and stored frozen at –70°C. Avoid multiple freeze-thaw cycles of frozen specimens.

Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before use. When possible avoid use of badly haemolysed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

7. Safety & precautions for use

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures , e.g.CDC/NIH Health manual : " Biosafety in Microbiological and Biomedical Laboratories" 1984
- Laboratory gloves should be worn at all times
- Avoid any skin contact with H_2SO_4 and TMB. In case of contact, wash thoroughly with water
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used
- Do not pipette by mouth
- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration
- Cover or cap all reagents when not in use
- Do not mix or interchange reagents between different lots
- Do not use reagents beyond the expiration date of the kit
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts
- Use a clean plastic container to prepare the washing solution
- Thoroughly mix the reagents and samples before use by agitation or swirling
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbance’s within 1 hour after completion of the assay
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells
- Follow incubation times described in the assay procedure
- Dispense the TMB solution within 15 min of the washing of the microtitre plate

8. Assay Preparation

Bring all reagents to room temperature before use

8.1. Assay Design

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard, zero and control should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the aluminium pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8^{o}C storage.

Example plate layout(example shown for a 6 point standard curve)

	Standards / Controls		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	500	500										
B	250	250										
C	125	125										
D	62.5	62.5										
E	31.25	31.25										
F	15.6	15.6										
G	zero	zero										
H	Ctrl	Ctrl										

All remaining empty wells can be used to test samples in duplicate

8.2. Preparation of Wash Buffer

Dilute the (200x) wash buffer concentrate 200 fold with distilled water to give a 1x working solution. Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder.Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2˚-25˚C.

8.3. Preparation of Standard Diluent Buffer

Add the contents of the vial (10x concentrate) to 225ml of distilled water before use.

This Solution can be stored at 2-8°C for up to 1 week.

8.4. Preparation of Standard

Depending on the type of samples you are assaying, the kit may include two standard diluents. Because biological fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure,you should reconstitute standard vials with the most appropriate StandardDiluent.

For **serum and plasma** samples: use Standard Diluent - Serum
For **cell culture supernatants**: use Standard Diluent Buffer

Standard vials must be reconstituted with the volume of standard diluent shown on the vial immediately prior to use. This reconstitution gives a stock solution of 500pg/ml of IL-1β. Mix the reconstituted standard gently by inversion only. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 500 to 15.6pg/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 200µl of the reconstituted standard to well's A1 and A2, which provides the highest concentration standard at 500pg/ml
- Add 100µl of appropriate standard diluent to the remaining standard wells B1 and B2 to F1 and F2
- Transfer 100µl from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells
- Continue this 1:1 dilution using 100µl from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 500pg/ml to 15.6pg/ml
- Discard 100µl from the final wells of the standard curve (F1 and F2)

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred directly into the relevant wells.

8.5. Preparation of Controls

Freeze-dried control vials should also be reconstituted with the most appropriate Standard Diluent to yoursamples.

For serum and plasma samples: use Standard Diluent- Serum
For cells culture supernatants: use Standard Diluent Buffer

The supplied Controls must be reconstituted with the volume of Standard Diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume, will give a solution at the concentration stated on the vial. Do not store after use.

8.6. Preparation of Biotinylated anti IL-1β

It is recommended this reagent is prepared immediately before use. Dilute the biotinylated anti IL-1β with the biotinylated antibody diluent in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

Number of wells required	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

8.7. Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.

Dilute the 5µl vial with 0.5ml of HRP diluent **immediately before use.** Do-not keep this diluted vial for future experiments. Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial. Please see example volumes below:

Number of wells required	Streptavidin-HRP (µl)	Streptavidin-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

9. Method

We strongly recommend that every vial is mixed thoroughly without foaming prior to use.

Prepare all reagents as shown in section 8.
Note: final preparation of Biotinylated Secondary Antibody (section 8.6) and Streptavidin-HRP (section 8.7) should occur immediately before use.

Assay Step		Details
1.	Addition	**Prepare Standard curve** as shown in section 8.4 above
2.	Addition	Add 100µl of each**, Sample, Standard, Control and zero (standard diluent)** in duplicate to appropriate number of wells
3.	Addition	Add 50µl of diluted **biotinylated anti IL-1β** to all wells
4.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **3 hour(s)**
5.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.3 ml of **1x washing solution** into each well c) Aspirate the contents of each well d) Repeat step b and c another two times
6.	Addition	Add 100µl of **Streptavidin-HRP** solution into all wells
7.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **30 min**
8.	Wash	Repeat wash step 5.
9.	Addition	Add 100µl of ready-to-use **TMB Substrate Solution** into all wells
10.	Incubation	Incubate in the dark for **10-20 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
11.	Addition	Add 100µl of **H_2SO_4:Stop Reagent** into all wells
Read the absorbance value of each well (immediately after step 11.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).		

***Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range*

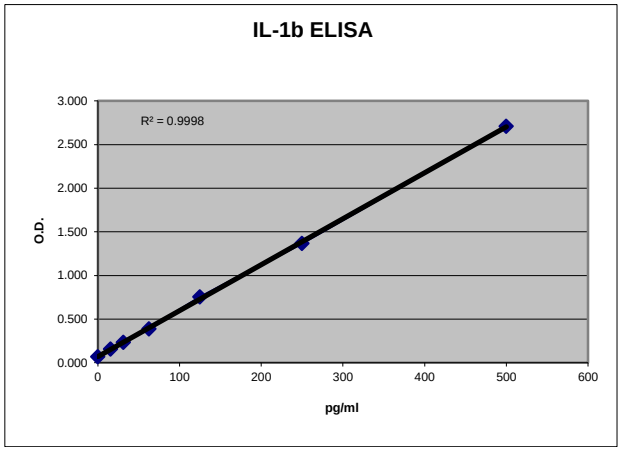
10. Data Analysis

Calculate the average absorbance values for each set of duplicate standards, controls and samples**.** Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding IL-1βstandard concentration on the horizontal axis.

The amount of IL-1βin each sample is determined by extrapolating OD values against IL-1βstandard concentrations using the standard curve**.**

Example of IL-1β Standard curve			
Standard	**IL-1β Conc**	**OD (450nm) mean**	**CV (%)**
1	500	2.708	4.1
2	250	1.368	3.6
3	125	0.767	2.6
4	62.5	0.410	7.4
5	31.25	0.262	16.5
6	15.6	0.162	5.9
zero	0	0.069	2.0



Note; curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

11. Assay limitations

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer, fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **afresh standard curve must be prepared and run for every assay.**

12. Performance Characteristics

12.1. Sensitivity

The sensitivity or minimum detectable dose of IL-1β using this OriGeneIL-1β ELISA kit was found to be **6.5 pg/ml**. This was determined by adding 2 standard deviations to the mean OD obtained when the zero standard was assayed in 6 independent experiments.

12.2. Specificity

The assay recognizes both natural and recombinant human IL-1β. To define the specificity of this ELISA several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested (IL-1α, IL-2, IL-10, IL-12, IL-17A, IL-23, IFNγ, Gp130, IL-33, TNFα).

12.3. Precision

Intra-assay

Reproducibility within the assay will be evaluated in three independent experiments. Each assay will be carried out with 6 replicates (3 duplicates) in 2 human pooled serum, 2 in RPMI and 2 in standard diluent with samples containing different concentrations of IL-1β. 2 standard curves were run on each plate **The overall intra-assay coefficient of variation has been calculated to be 4.5% .**

Session	***Sample***	**Mean** IL-1β pg/ml	**SD**	**7**
Session 1	***Sample 1***	415.68	20.97	**5.05**
	Sample 2	273.72	14.08	**5.14**
	Sample 3	420.93	21.41	**5.09**
	Sample 4	186.50	12.12	**6.50**
	Sample 5	443.97	11.72	**2.64**
	Sample 6	323.87	14,08	**4.35**
Session 2	***Sample 1***	437.48	8.27	**1.89**
	Sample 2	282.31	14.84	**5.26**
	Sample 3	428.22	44.91	**10.49**
	Sample 4	203.00	10.15	**5.00**
	Sample 5	471.06	13.01	**2.76**
	Sample 6	325.11	22.73	**6.99**
Session 3	***Sample 1***	399.39	10.28	**2.57**
	Sample 2	279.55	11.09	**3.94**
	Sample 3	387.02	7.10	**1.83**
	Sample 4	182.91	5.02	**2.74**
	Sample 5	430.74	28.82	**6.69**
	Sample 6	323.79	9.35	**2.89**

Inter-assay

Assay to assay reproducibility within one laboratory will be evaluated in three independent experiments by two technicians. Each assay will be carried out with 6 replicates (3 duplicates) in 2 human pooled serum, 2 in RPMI and 2 in standard diluent with samples containing different concentrations of IL-1β. 2 standard curves were run on each plate. **The calculated overall coefficient of variation was 8.7%.**

	Sample 1	***Sample2***	***Sample 3***	***Sample 4***	***Sample 5***	***Sample 6***
Mean IL-1β pg/ml	410	273	402	184	445	314
SD	30	26	33	22	26	31
CV	**7.4**	**9.5**	**8.2**	**11.7**	**5.7**	**9.9**

12.4. Dilution Parallelism

Three spiked human serum samples with different levels of IL-1βwere analysed at different serial two fold dilutions (1:2 To 1:16) with two replicates each. Recoveries ranged from 83 to 111% with an overall **mean recovery of 96%.**

12.5. Spike Recovery

The spike recovery was evaluated by spikingin human serum and culture medium samples different concentrations of IL-1β in 3 separate experiments. Recoveries ranged from 72 to 105% with an overall **mean recovery of 89%**

12.6. Stability

Storage Stability

Aliquots of spiked serum and spiked medium were stored at –20°C, 4°C, room temperature (RT) and at 37°C and the IL-1βlevel determined after 24h. We observed between 20 and 30% loss when stored at 4°C, RT and 37°C when spiked in serum. When spiked in culture media, we observed no loss at 4°C and RT, but up to 60% loss at 37°C.

Freeze-thaw Stability

Aliquots of spiked serum and spiked medium were stored frozen at –20°C and thawed up to 5 times and the IL-1βlevel was determined. We observed around 20% loss after 3 cycles and up to 30% after 5 cycles when spiked in serum. No loss observed when spiked in cell culture media.

12.7. Expected serum values

A panel of 20 human sera and 20 Plasma samples were tested for IL-1β. All were evaluated below the detection level (6,5pg/ml).

12.8. Standard Calibration (NIBSC Calibration)

This immunoassay is calibrated against the International Reference Standard NIBSC 86/680 IL-1β. NIBSC 01/420 is quantitated in International Units (IU). 1IU (approximately 100pg) has been showed to be equivalent to 90 pg OriGeneIL-1β.

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15. Assay Summary

Total procedure length: 3h45mn

Add 100 µl of sample and diluted standard/controls and 50µl Biotinylated anti IL-1β

↓

Incubate 3 hours at room temperature

↓

Wash three times

↓

Add 100µl of Streptavidin-HRP

↓

Incubate 30min at room temperature

↓

Wash three times

↓

Add 100µl of ready-to-use TMB
Protect from light. Let the color develop for 12-15 mn.

↓

Add 100µl H_2SO_4

↓

Read Absorbance at 450 nm

TECHNICAL CONSULTATION

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