abcam

Product datasheet

Human IL-1 beta ELISA Kit ab214025

KO VALIDATED Recombinant

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Overview

Product name Human IL-1 beta ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Overall	8			4.8%

Inter-assay

Sample	n	Mean	SD	CV%
Overall	3			5.6%

Sample type Cell culture supernatant, Serum, Hep Plasma, EDTA Plasma, Cit plasma

Assay type Sandwich (quantitative)

Sensitivity 5.64 pg/ml

Range 14.06 pg/ml - 900 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture supernatant	98	96% - 100%
Serum	103	101% - 105%
Hep Plasma	100	99% - 101%
EDTA Plasma	93	90% - 96%
Cit plasma	86	84% - 88%

Assay time 2h 30m

Assay duration

One step assay

Species reactivity

Reacts with: Human
Does not react with: Cow

Product overview

Human IL-1 beta (Interleukin 1 beta) ELISA kit is a single-wash sandwich ELISA designed for the quantitative measurement of IL-1 beta protein in human serum, plasma, and cell culture supernatants. It uses our proprietary SimpleStep ELISA® technology. Quantitate human IL-1 beta with 5.64 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (**ab203359**) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

ASSAY SPECIFICITY

This kit recognizes both native and recombinant human IL-1beta protein in serum, plasma, and cell culture supernatant samples only.

Cell and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY

Recombinant mouse IL-1beta and human IL-1 receptor antagonist were prepared at 50 ng/mL and 225 pg/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant human Interleukin-1 receptor type 1 was prepared at 50 ng/mL and 225 pg/mL and tested for interference. No interference with was observed.

SPECIES REACTIVITY

This kit recognizes human IL-1beta protein.

Other species reactivity was determined by measuring serum samples of various species, interpolating the IL-1beta protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the IL-1beta protein concentration in human

serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

Other species reactivity not determined.

Notes Interleukin 1 beta (IL-1 beta) is produced by activated macrophages and stimulates thymocyte proliferation by inducing IL-2 release, B-cell maturation and proliferation, and fibroblast growth factor activity. IL-1 proteins are involved in the inflammatory response, being identified as endogenous pyrogens, and are reported to stimulate the release of prostaglandin and collagenase from synovial cells.

Platform Pre-coated microplate (12 x 8 well strips)

Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	1 x 96 tests	10 x 96 tests
10X Human IL-1beta Capture Antibody	1 x 600µl	10 x 600µl
10X Human IL-1beta Detector Antibody	1 x 600µl	10 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml	1 x 200ml
Antibody Diluent 4BI	1 x 6ml	10 x 6ml
Human IL-1beta Lyophilized Recombinant Protein (ab9617)	2 vials	2 x 10 vials
Plate Seals	1 unit	10 units
Sample Diluent NS (ab193972)	1 x 50ml	2 x 250ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit	10 units
Stop Solution	1 x 12ml	1 x 120ml
TMB Development Solution	1 x 12ml	1 x 120ml

Function Potent proinflammatory cytokine. Initially discovered as the major endogenous pyrogen, induces prostaglandin synthesis, neutrophil influx and activation, T-cell activation and cytokine production, B-cell activation and antibody production, and fibroblast proliferation and collagen production. Promotes Th17 differentiation of T-cells.

Tissue specificity Expressed in activated monocytes/macrophages (at protein level).

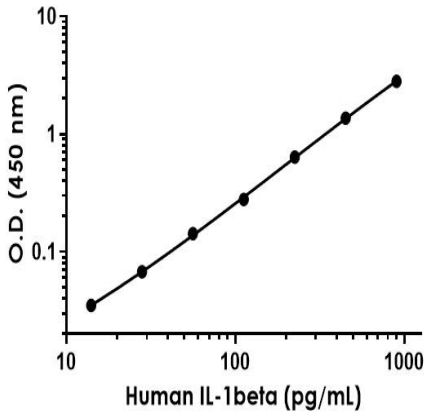
Sequence similarities Belongs to the IL-1 family.

Post-translational modifications Activation of the IL1B precursor involves a CASP1-catalyzed proteolytic cleavage. Processing and secretion are temporarily associated.

Cellular localization Cytoplasm, cytosol. Lysosome. Secreted, exosome. Cytoplasmic vesicle, autophagosome. Secreted. The precursor is cytosolic. In response to inflammasome-activating signals, such as ATP for NLRP3 inflammasome or bacterial flagellin for NLRC4 inflammasome, cleaved and

secreted. IL1B lacks any known signal sequence and the pathway(s) of its secretion is(are) not yet fully understood (PubMed:24201029). On the basis of experimental results, several unconventional secretion mechanisms have been proposed. 1. Secretion via secretory lysosomes: a fraction of CASP1 and IL1B precursor may be incorporated, by a yet undefined mechanism, into secretory lysosomes that undergo Ca(2+)-dependent exocytosis with release of mature IL1B (PubMed:15192144). 2. Secretory autophagy: IL1B-containing autophagosomes may fuse with endosomes or multivesicular bodies (MVBs) and then merge with the plasma membrane releasing soluble IL1B or IL1B-containing exosomes (PubMed:24201029). However, autophagy impacts IL1B production at several levels and its role in secretion is still controversial. 3. Secretion via exosomes: ATP-activation of P2RX7 leads to the formation of MVBs containing exosomes with entrapped IL1B, CASP1 and other inflammasome components. These MVBs undergo exocytosis with the release of exosomes. The release of soluble IL1B occurs after the lysis of exosome membranes (By similarity). 4. Secretion by microvesicle shedding: activation of the ATP receptor P2RX7 may induce an immediate shedding of membrane-derived microvesicles containing IL1B and possibly inflammasome components. The cytokine is then released in the extracellular compartment after microvesicle lysis (PubMed:11728343). 5. Release by translocation through permeabilized plasma membrane. This may occur in cells undergoing pyroptosis due to sustained activation of the inflammasome (By similarity). These mechanisms may not be not mutually exclusive.

Images



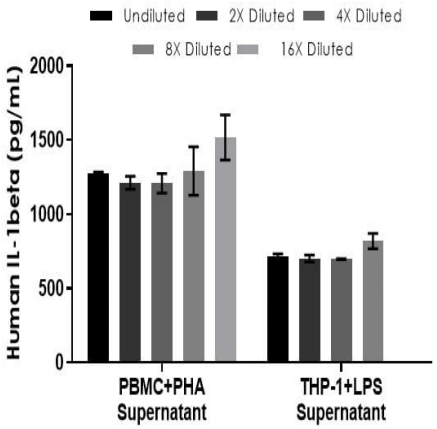
Example of human IL-1beta standard curve.

Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.065	0.066	0.066
14.06	0.101	0.100	0.101
28.13	0.134	0.133	0.133
56.25	0.209	0.208	0.208
112.50	0.345	0.341	0.343
225	0.714	0.694	0.704
450	1.383	1.479	1.431
900	2.822	2.907	2.864

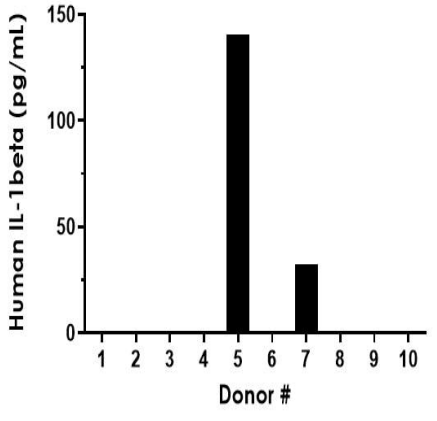
Standard curve

Example of human IL-1beta standard curve in Sample Diluent NS. The IL-1beta standard curve was prepared as described. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



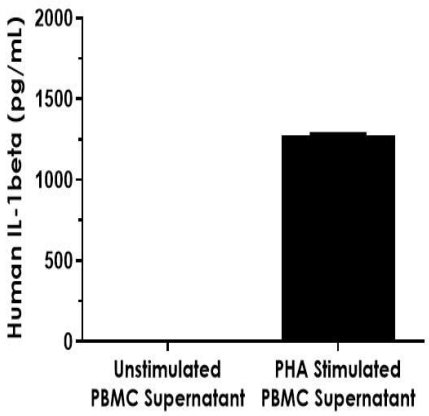
Interpolated concentrations of native IL-1beta in human PHA stimulated PBMC supernatant and LPS stimulated THP-1 supernatant samples.

The concentrations of IL-1beta were measured in duplicates, interpolated from the IL-1beta standard curves and corrected for sample dilution. Undiluted samples are as follows: PBMC supernatant 50% and 100% THP-1 supernatant. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean IL-1beta concentration was determined to be 1301 pg/mL in PBMC supernatant and 734 pg/mL in THP-1 supernatant.



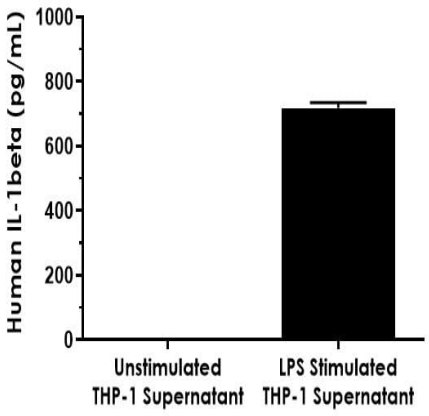
Serum from ten individual healthy human male donors was measured in duplicate.

Interpolated values are plotted (mean +/- SD, n=2). IL-1beta was measured in 2 donor serum samples (30 pg/mL and 140 pg/mL) and the remaining 8 samples measured less than the lowest point of the IL-1beta standard curve.



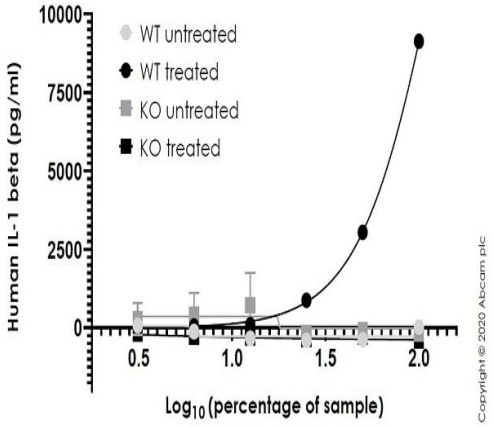
Human peripheral blood mononuclear cells were cultured unstimulated or stimulated with 10 µg/mL PHA.

Conditioned media was harvested after 48 hours. IL-1beta was measured in 50% unstimulated and PHA stimulated PBMC supernatant. The concentrations of IL-1beta were measured in duplicate, interpolated from the IL-1beta standard curves and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean IL-1beta concentration was determined to be 1273 pg/mL in PHA stimulated PBMC supernatant. There was no detectable signal in unstimulated supernatant.



THP-1 cells were cultured unstimulated or stimulated with 5 µg/mL Lipopolysaccharide (LPS).

Conditioned media was harvested after 48 hours. IL-1beta was measured in 100% unstimulated and LPS stimulated THP-1 supernatant. The concentrations of IL-1beta were measured in duplicate and interpolated from the IL-1beta standard curves. The interpolated values are plotted (mean +/- SD, n=2). The mean IL-1beta concentration was determined to be 718 pg/mL in LPS stimulated THP-1 supernatant. There was no detectable signal in unstimulated supernatant.



Sandwich ELISA - Human IL-1 beta ELISA Kit, Fluorescent (**ab229384**)

This data was collected using **ab229384**, which uses the same recombinant antibody pair and standard protein. Human IL-1 beta concentration was interpolated from the standard curve. Supernatants from cell culture samples were serially diluted and assessed by the Human IL-1 beta ELISA kit (**ab229384**). Wild-type and IL-1 beta knockout THP-1 cells (**ab273762**) were assessed in duplicate (n=2). Cells were either treated with LPS (100 ng/ml, 3 h) then ATP (5 mM, 45 min) to induce expression of IL-1 beta or not treated. Data are represented as the mean and error bars represent standard deviation.

Dilution Factor	Interpolated value	50% PHA Stimulated Supernatant	100% LPS Stimulated THP-1 Supernatant
Undiluted	pg/mL	637	718
	% Expected value	100	100
2	pg/mL	303	351
	% Expected value	95	98
4	pg/mL	151	174
	% Expected value	95	97
8	pg/mL	81	102
	% Expected value	101	114
16	pg/mL	47	NL
	% Expected value	119	NL

NL – Non-Linear

Linearity of dilution.

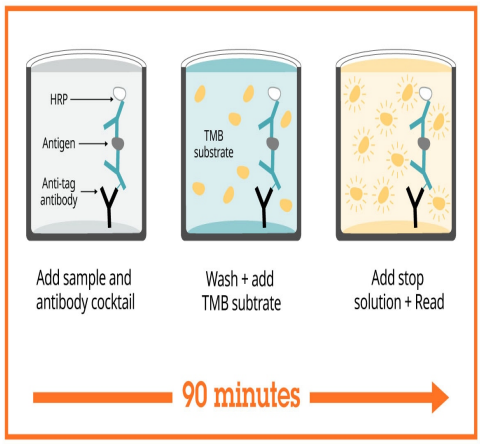
Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native IL-1beta was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Serum	50% Human Plasma (Citrate)	50% Human Plasma (Heparin)	50% Human Plasma (EDTA)
Undiluted	pg/mL	444	385	445	451
	% Expected value	100	100	100	100
2	pg/mL	232	207	228	221
	% Expected value	104	107	103	98
4	pg/mL	114	105	116	115
	% Expected value	103	109	104	102
8	pg/mL	56	53	57	58
	% Expected value	101	110	103	103
16	pg/mL	24	26	27	27
	% Expected value	87	107	96	97

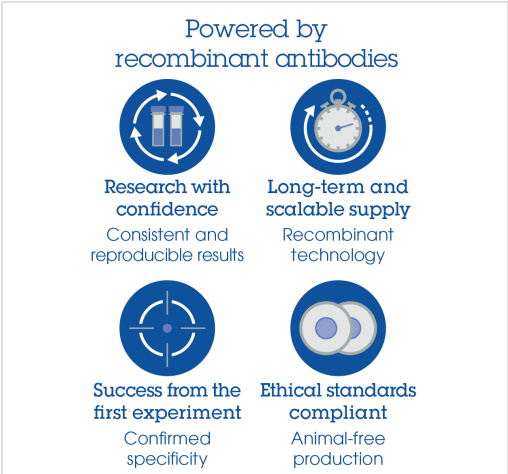
Linearity of dilution.

Recombinant IL-1beta was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.



Sandwich ELISA - Human IL-1 beta ELISA Kit (ab214025)

SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



Sandwich ELISA - Human IL-1 beta ELISA Kit (ab214025)

To learn more about the advantages of recombinant antibodies see **here**.

Sandwich ELISA - Human IL-1 beta ELISA Kit (ab214025)

To learn more about the advantages of SimpleStep ELISA® kits see **here**.

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