

Product datasheet

Human IL-1 beta ELISA Kit, Fluorescent ab229384

KO VALIDATED Recombinant

6 References 7 Images

Overview							
Product name	Human IL-1 beta ELISA Kit, Fluorescent						
Detection method	Fluorescent						
Precision							Intra-assay
	Sample	n	Mea	in	SD		CV%
	Media	8					4.8%
							Inter-assay
	Sample	n	Mea	in	SD		CV%
	Media	3					5.6%
Sample type	Cell culture supernatant, Serum, Hep Plasma, EDTA Plasma, Cit plasma						
Assay type	Sandwich (quantitative)						
Sensitivity	2.6 pg/ml						
Range	3.5 pg/ml - 3600 pg/ml						
Recovery						;	Sample specific recovery
	Sample type			Average %		Rang	ge
	Cell culture supernatant			98		96%	- 100%
	Serum			103		101%	% - 105%

100

93

86

2h 00m

Hep Plasma

EDTA Plasma

Cit plasma

99% - 101%

90% - 96%

84% - 88%

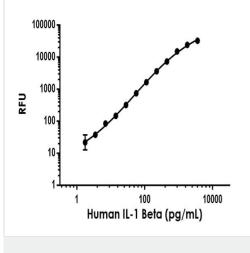
Assay duration	One step assay
Species reactivity	Reacts with: Human Does not react with: Cow
Product overview	IL-1 beta <i>in vitro</i> CatchPoint [®] ELISA kit is designed for the quantitative measurement of IL-1 beta protein in human serum, plasma and cell culture supernatants.
	This CatchPoint ELISA kit has been optimized for Molecular Devices Microplate Readers . Click <u>here</u> for a list of recommended Microplate Readers. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint ELISA Kits is available with all the protocol and analysis settings at <u>www.softmaxpro.org</u> .
	The CatchPoint [®] ELISA employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. CatchPoint HRP Development Solution containing the Stoplight Red Substrate is added. During incubation, the substrate is catalyzed by HRP generating a fluorescent product. Signal is generated proportionally to the amount of bound analyte and the intensity is measured in a fluorescence plater reader at 530/570/590 nm Excitation/Cutoff/Emission.
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

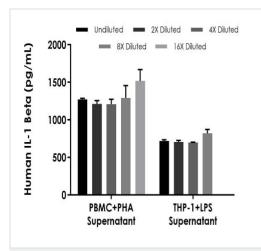
Storage instructions	Store at +4°C. Please refer to protocols.	
Components		1 x 96 tests
100X Stoplight Red Substrate		1 x 120µl
10X Human IL-1beta Capture Antibody		1 x 600µl
10X Human IL-1beta Detector Antibody		1 x 600µl
10X Wash Buffer PT (ab206977)		1 x 20ml
500X Hydrogen Peroxide (H2O2, 3%)		1 x 50µl
Antibody Diluent 4BI		1 x 6ml
Human IL-1beta Lyophilized Recombinant Protein (ab9617)		2 vials
Plate Seal		1 unit

Components	1 x 96 tests
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated Black 96-Well Microplate	1 unit
Stoplight Red Substrate Buffer	1 x 12ml

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.



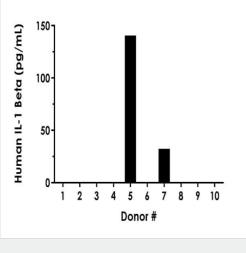
Example of human IL-1 beta standard curve in Sample Diluent NS.



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

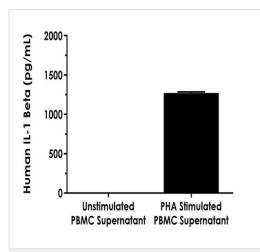
The IL-1 beta standard curve was prepared as described in Section 10. Background-subtracted data values (mean +/- SD) are graphed.

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.



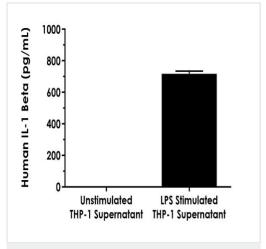
Interpolated values are plotted (mean +/- SD, n=2). IL-1beta was measured in 2 donor serum samples (30 pg/mL and 140 pg/mL) and the remaining 8 samples measured less than the lowest point of the IL-1beta standard curve.

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

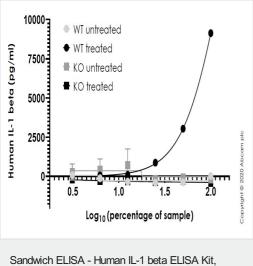


Human peripheral blood mononuclear cells were cultured unstimulated or stimulated with 10 $\mu\text{g/mL}$ PHA.

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).



Fluorescent (ab229384)

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.

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.



To learn more about the advantages of recombinant antibodies see <u>here</u>.

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