ab327306 – Rat IL-1 beta SimpleStep ELISA® Kit Chemiluminescent

For the quantitative measurement of IL-1 beta in rat serum, plasma (EDTA), plasma (citrate), and cell culture supernatant.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab327306

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve, sensitivity and native serum and plasma (citrate, EDTA) values were collected using the colorimetric version of this kit (ab255730).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Rat IL-1 beta Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Rat IL-1 beta Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Rat IL-1 beta Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4BR	6 mL	10 x 6 mL	+4°C
Sample Diluent 75BP	20 mL	10 x 20 mL	+4°C
Sample Diluent NS	12 mL	2 x 50 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Luminometer capable of reading strip plates with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent 75BP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BR. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BR. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the **IL-1 beta** protein standard by adding the volume indicated on the protein vial label. For **serum and plasma samples measurements**, use Sample Diluent 75BP. For **cell culture supernatant samples measurements**, use Sample Diluent NS. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 12 ng/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 75 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	375	75	12,000	10,000
2	Standard#1	75	150	10,000	3,333.33
3	Standard#2	75	150	3,333.33	1,111.11
4	Standard#3	75	150	1,111.11	370.37
5	Standard#4	75	150	370.37	123.46
6	Standard#5	75	150	123.46	41.15
7	Standard#6	75	150	41.15	13.72
8	Standard#7	75	150	13.72	4.57
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	≤ 50%
Plasma – Citrate*	≤ 50%
Plasma – EDTA*	≤ 50%
1640 RPMI + 10% FBS Cell Culture Media*	≤ 50%

*Based on spiked sample in absence of native signal.

Serum:
Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1: 2 into Sample Diluent 75BP and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

Plasma:
Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1: 2 into Sample Diluent 75BP and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma (heparin) samples.

Cell Culture Supernatants:
Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1: 2 into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4ºC.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support
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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of rat IL-1 beta.

The standard protein in this kit is full-length mature rat IL-1 beta.

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), and cell culture supernatant sample types.

50% pooled serum and plasma (citrate and EDTA) samples from healthy donors was measured in duplicate. All values were below the detectable range of the assay.

Urine, saliva, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

This kit is incompatible with plasma (heparin) samples.

SPECIES REACTIVITY

This kit recognizes rat IL-1 beta protein.

Recombinant mouse and human IL-1 beta were each prepared at 50 ng/mL and 2,400 pg/mL and assayed for cross reactivity. No reactivity was observed.

50% serum samples of various other species were measured in duplicates. Interpolated values were below the detectable range of the assay for the following species: Human, Mouse, Cow.

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0.00	445	696	571
4.57	1,636	1,032	1,334
13.72	1,951	1,910	1,931
41.15	5,371	6,963	6,167
123.46	17,466	17,059	17,263
370.37	53,198	50,599	51,899
1,111.11	157,200	150,610	153,905
3,333.33	371,330	364,530	367,930
10,000.00	816,580	837,350	826,965

Table 1. Example of rat IL-1 beta standard curve in Sample Diluent 75BP. The IL-1 beta standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0.00	345	497	421
4.57	984	883	934
13.72	1,541	1,600	1,571
41.15	4,817	4,121	4,469
123.46	11,900	12,882	12,391
370.37	45,770	45,374	45,572
1,111.11	142,060	143,250	142,655
3,333.33	359,420	362,370	360,895
10,000.00	764,220	771,150	767,685

Table 2. Example of rat IL-1 beta standard curve in Sample Diluent NS. The IL-1 beta standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent 75BP	16	2.98 pg/mL
Sample Diluent NS	16	2.92 pg/mL

Recovery

Three concentrations of IL-1 beta were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Serum (50%)	90	82 – 94
Plasma – Citrate (50%)	91	85 – 97
Plasma – EDTA (50%)	85	81 – 88
Cell culture Media* (50%)	108	103 – 112

*Media is RPMI 1640 containing 10% fetal bovine serum.

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.

Recombinant IL-1 beta was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 75BP.

Dilution Factor	Interpolated value	50% Serum	50% Plasma (Citrate)	50% Plasma (EDTA)
Undiluted	pg/mL	1,241	1,335	1,355
	% Expected value	100	100	100
2	pg/mL	652	667	717
	% Expected value	105	100	106
4	pg/mL	362	360	391
	% Expected value	117	108	115
8	pg/mL	173	180	198
	% Expected value	112	108	117
16	pg/mL	85	87	102
	% Expected value	109	104	121

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

Dilution Factor	Interpolated value	50% 1640 RPMI + 10% FBS
Undiluted	pg/mL	1,205
	% Expected value	100
2	pg/mL	577
	% Expected value	96
4	pg/mL	293
	% Expected value	97
8	pg/mL	147
	% Expected value	97
16	pg/mL	71
	% Expected value	94

Precision

Mean coefficient of variations of interpolated values of IL-1 beta from three concentrations of recombinant standard protein within the working range of the assay spiked into rat serum (50%).

	Intra-assay	Inter-assay
N=	8	3
CV (%)	3.9	4.3

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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