

## ab326301 – Rat IL-10 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of IL-10 in rat serum, 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/ab326301](http://www.abcam.com/ab326301)

**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 and sensitivity (were collected using the colorimetric version of this kit (ab214566).

### Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Rat IL-10 Capture Antibody 10X	600 µL	+4°C
Rat IL-10 Detector Antibody 10X	600 µL	+4°C
Rat IL-10 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BR	6 mL	+4°C
Sample Diluent 25BS	20 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

### Materials Required, Not Supplied

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

Luminometer 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 25BS 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 5BR. 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 5BR. 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-10** protein standard by adding the volume indicated on the protein vial label. For **cell culture supernatant samples measurements**, use Sample Diluent NS. For **serum and plasma samples measurements**, use Sample Diluent 25BS. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 6,000 pg/mL **Stock Standard #1** 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 150 µL of Sample Diluent into numbers 2-9.
4. Use the **Stock Standard #1** 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 #1	225	0	6,000	6,000
2	Stock Standard #1	75	150	6,000	2,000
3	Standard#2	75	150	2,000	666.7
4	Standard#3	75	150	666.7	222.2
5	Standard#4	75	150	222.2	74.1
6	Standard#5	75	150	74.1	24.7
7	Standard#6	75	150	24.7	8.2
8	Standard#7	75	150	8.2	2.7
9	Blank Control	0	150	0	0

### Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	1.56 – 25%
Plasma – Citrate	3.13 – 50%
Cell Culture Supernatant	Varies by type

**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 1:4 into Sample Diluent 25BS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

**Plasma** Collect plasma using citrate. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:2 into Sample Diluent 25BS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Cell Culture Supernatants** Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C 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 4°C.

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 4°C 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.

<b>Mode:</b>	Luminescence
<b>Instrument settings:</b>	Endpoint
<b>Detection Mode:</b>	All wavelengths
<b>Read Time:</b>	0.5-1 sec
<b>Read:</b>	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

Copyright © 2026 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

Version 1 | 2026-03-12

For all technical or commercial enquiries please go to:

<https://www.abcam.com/en-us/contact-us>

<https://www.abcam.cn/contact-us> (China)

<https://www.abcam.co.jp/contact-us> (Japan)

# ab326301 – Rat IL-10 SimpleStep ELISA® Kit, Chemiluminescent

## Additional information

### ASSAY SPECIFICITY

This kit is designed for the quantification of rat IL-10.

The standard protein in this kit is full-length mature rat IL-10.

Native signal was detected in serum, plasma (citrate), and cell culture supernatant sample types.

Plasma (EDTA), plasma (heparin), urine, saliva, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

### SPECIES REACTIVITY

Recombinant human and mouse IL-10 were prepared at 50 ng/mL and 1 ng/mL and were assayed for cross reactivity. No cross reactivity was observed.

Other species reactivity was determined by measuring 25% serum samples of various species, interpolating the protein concentrations from the rat standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in rat serum assayed at the same dilution.

Reactivity <3% was determined 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	338	732	535
2.7	1,639	1,563	1,601
8.2	4,089	3,739	3,914
24.7	11,903	11,951	11,927
74.1	45,734	43,215	44,475
222.2	143,970	143,170	143,570
666.7	396,440	394,670	395,555
2,000	1,150,500	1,094,000	1,122,250
6,000	3,563,500	3,241,000	3,402,250

Table 1. Example of rat IL-10 standard curve in Sample Diluent NS. The IL-10 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	555	1,043	799
2.7	1,729	1,809	1,769
8.2	2,792	2,642	2,717
24.7	6,679	6,856	6,768
74.1	20,879	21,347	21,113
222.2	68,096	71,831	69,964
666.7	205,090	203,670	204,380
2,000	651,660	633,930	642,795
6,000	2,108,700	2,059,300	2,084,000

Table 2. Example of rat IL-10 standard curve in Sample Diluent 25BS. The IL-10 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 NS	16	0.8 pg/mL
Sample Diluent 25BS	16	1.45 pg/mL

### Recovery

Three concentrations of IL-10 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 (%)
25% Serum	94	93 - 94
50% Plasma - Citrate	86	85 - 89
50% PBMC Supernatant	93	84 - 109

### 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-10 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% PBMC + PHA Supernatant	100% PBMC Supernatant	50% Spleen + Concanavalin A Supernatant
Undiluted	pg/mL	534	213	361
	% Expected value	100	100	100
2	pg/mL	281	114	207
	% Expected value	105	107	115
4	pg/mL	139	62	102
	% Expected value	104	116	113
8	pg/mL	79	NL	NL
	% Expected value	119	NL	NL
16	pg/mL	40	NL	NL
	% Expected value	121	NL	NL

NL - Non-Linear

Recombinant IL-10 was spiked into the following biological samples and diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 25BS.

Dilution Factor	Interpolated value	25% Rat Serum	50% Rat Plasma (Citrate)
Undiluted	pg/mL	1,708	1,968
	% Expected value	100	100
2	pg/mL	837	899
	% Expected value	98	91
4	pg/mL	401	458
	% Expected value	94	93
8	pg/mL	220	221
	% Expected value	103	90
16	pg/mL	103	110
	% Expected value	97	89

### Precision

Mean coefficient of variations of interpolated values of IL-10 from three concentrations of rat PHA-stimulated PBMC supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	5.6	3.4

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

Copyright © 2026 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

Version 1 | 2026-03-12

For all technical or commercial enquiries please go to:

<https://www.abcam.com/en-us/contact-us>

<https://www.abcam.cn/contact-us> (China)

<https://www.abcam.co.jp/contact-us> (Japan)