ab325446 – Human IL-10 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of IL-10 in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.
For research use only - not intended for diagnostic use.
Patent pending.

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 the individual donors were collected using the colorimetric version of this kit (ab185986).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human IL-10 Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human IL-10 Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human IL-10 Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent CPI2	6 mL	10 x 6 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	10 x 1 mL	+4°C
Sample Diluent NBP	20 mL	10 x 20 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 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 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.
Plate shaker for all incubation steps.
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 NBP 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.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

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.

Sample Diluent 50BP: Prepare Sample Diluent 50BP by thoroughly mixing Sample Diluent NBP, then diluting Sample Diluent NBP with 1X Wash Buffer PT. To make 2 mL Sample Diluent 50BP, combine 1 mL 1X Wash Buffer PT and 1 mL Sample Diluent NBP. Mix thoroughly by vortexing and heating gently in a warm water bath until debris is completely dissolved. If required, protease inhibitors can be added.

Sample Diluent 50BP + 1X Enhancer: Prepare Sample Diluent 50BP + Enhancer by thoroughly mixing Sample Diluent NBP, then diluting Sample Diluent NBP and 50X Cell Extraction Enhancer Solution with 1X Wash Buffer PT. To make 5 mL Sample Diluent 50BP + Enhancer, combine 2.4 mL 1X Wash Buffer PT, 2.5 mL Sample Diluent NBP, and 100 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly by vortexing and heating gently in a warm water bath until debris is completely dissolved. If required, protease inhibitors can be added.

Sample Diluent NS + 1X Enhancer: Prepare Sample Diluent NS + 1X Enhancer by combining Sample Diluent NS and 50X Cell Extraction Enhancer Solution. To make 5 mL Sample Diluent NS + 1X Enhancer, combine 4.9 mL Sample Diluent NS and 100 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. 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 CPI2. 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 **serum** and **plasma sample measurements**, use Sample Diluent 50BP + 1X Enhancer. For **cell culture supernatant sample measurements**, use Sample Diluent NS + 1X Enhancer. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 11,550 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 141 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 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**	90	141	11,550	4,500
2	Standard#1	75	150	4,500	1,500
3	Standard#2	75	150	1,500	500
4	Standard#3	75	150	500	166.7
5	Standard#4	75	150	166.7	55.6
6	Standard#5	75	150	55.6	18.5
7	Standard#6	75	150	18.5	6.2
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	≤ 50%
Plasma – Citrate*	≤ 50%
Plasma – EDTA*	≤ 25%
Plasma – Heparin*	≤ 25%
1.5% PHA-Stimulated PBMC Cell Culture Supernatant	3.1 – 50%

***Based on spiked sample

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. To 100 µL serum sample add 96 µL Sample Diluent 50BP **(without enhancer)** and 4 µL 50X Cell Extraction Enhancer Solution. Assay, or dilute samples into Sample Diluent 50BP + 1X Enhancer and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. To 100 µL plasma sample add 96 µL Sample Diluent 50BP **(without enhancer)** and 4 µL 50X Cell Extraction Enhancer Solution. Dilute plasma (EDTA) and plasma (heparin) samples at least 2-fold further into Sample Diluent 50BP + 1X Enhancer and assay. Assay plasma (citrate) samples or dilute the samples further into Sample Diluent 50BP + 1X Enhancer and assay. Store un-diluted serum at -20° or below. 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. To 100 mL sample add 96 mL Sample Diluent NS **(without enhancer)** and 4 mL 50X Cell Extraction Enhancer Solution. Assay or dilute the samples further into Sample Diluent NS + 1X Enhancer 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.

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:
www.abcam.com/protocols/the-complete-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.

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)

Copyright © 2026 Abcam, All Rights Reserved. All information / detail is correct at time of going to print.
Version 2 | 2026-07-08

ab325446 – Human IL-10 SimpleStep ELISA® Kit, Chemiluminescent

Additional information

ASSAY SPECIFICITY

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

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

Native signal was detected in cell culture supernatant.

Spiked protein experiments were used to validate serum, plasma (heparin), plasma (EDTA), plasma (citrate) sample types.

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

50% serum from 8 individual healthy human female donors was measured in duplicate. All values were below the detectable range of the assay.

Saliva, urine, milk, CSF, cell extract, tissue extract samples have not been tested with this kit.

INTERFERENCE

Recombinant human IL-10 R alpha and human IL-10 R beta were each prepared at 50 ng/mL and 0.6 ng/mL and tested for interference. No interference with was observed.

SPECIES REACTIVITY

Recombinant mouse and rat IL-10 were prepared at 50 ng/mL and 0.6 ng/mL each and assayed for cross reactivity. No cross-reactivity was observed.

Other species reactivity was determined by measuring 50% serum samples of various species, interpolating the protein concentrations from the human standard curve.

Interpolated values were below the detectable range of the assay for the following species: Mouse, Rat, Cow.

Other species reactivity not determined.

CALIBRATION

This immunoassay is calibrated against a highly purified human IL-10. The NIBSC/WHO unclassified purified human IL-10 preparation 93/722 was evaluated in this kit.

The dose response curve of the unclassified standard IL-10 parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep human IL-10 kit to approximate NIBSC 93/722 units, use the equation below.

NIBSC (93/722) approximate value (IU/mL) = 0.01236 x SimpleStep Human IL-10 value (pg/mL).

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	868	927	898
6.2	1,968	1,975	1,972
18.5	5,436	5,371	5,404
55.6	16,130	16,319	16,225
166.7	57,206	62,629	59,918
500	209,370	227,520	218,445
1,500	860,790	865,660	863,225
4,500	2,579,700	2,560,500	2,570,100

Table 1. Example of human IL-10 standard curve in Sample Diluent 50BP+ Enhancer. 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	536	603	570
6.2	7,109	7,365	7,237
18.5	21,237	22,438	21,838
55.6	76,946	78,164	77,555
166.7	263,720	271,440	267,580
500	734,890	845,930	790,410
1,500	2,242,100	2,474,600	2,358,350
4,500	4,557,800	5,123,600	4,840,700

Table 2. Example of human IL-10 standard curve in Sample Diluent NS+ Enhancer. 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 50BP + 1X Enhancer	8	2.9 pg/mL
Sample Diluent NS + 1X Enhancer	8	1.0 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 (%)
50% Serum	109	108 - 112
50% Plasma – Citrate	111	105 - 119
25% Plasma – EDTA	109	103 - 118
25% Plasma – Heparin	116	113 - 117
25% Stimulated PBMC Cell Cutlure Supernatant*	108	105 - 111
95% Cell Culture Media*	106	102 - 111

*Media is RPMI 1640 containing 10% fetal calf 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-10 was spiked into the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 50BP+ 1X Enhancer.

Dilution Factor	Interpolated value	50% Serum	50% Plasma (Citrate)	25% Plasma (EDTA)	25% Plasma (Heparin)
Undiluted	pg/mL	2,410	2,293	2,279	2,110
	% Expected value	100	100	100	100
2	pg/mL	1,078	1,052	1,079	1,042
	% Expected value	89	92	95	99
4	pg/mL	526	527	531	523
	% Expected value	87	92	93	99
8	pg/mL	243	260	251	266
	% Expected value	81	91	88	101
16	pg/mL	118	113	126	123
	% Expected value	83	87	88	93

Native IL-10 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS + 1X Enhancer.

Dilution Factor	Interpolated value	50% 1.5% PHA-Stimulated PBMC SN
Undiluted	pg/mL	227
	% Expected value	100
2	pg/mL	119
	% Expected value	105
4	pg/mL	59
	% Expected value	103
8	pg/mL	29
	% Expected value	101
16	pg/mL	14
	% Expected value	101

Precision

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

	Intra-assay	Inter-assay
N=	5	3
CV (%)	5.2	3.2

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-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.

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)

Copyright © 2026 Abcam, All Rights Reserved. All information / detail is correct at time of going to print.
Version 2 | 2026-07-08