

ab325448 – Human IL-6 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of IL-6 in human serum, plasma (citrate), plasma (EDTA), cell culture supernatant, urine, and CSF

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: Typical standard curve, sensitivity, and urine sample validation were determined using Chemiluminescent detection. Unless stated, all remaining data was collected using the colorimetric version of this kit (ab178013).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human IL-6 Lyophilized Capture Antibody	1 Vial	10 x 1 Vial	+4°C
Human IL-6 Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human IL-6 Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 5BI	6 mL	10 x 6 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.

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.

10X Capture Antibody: To reconstitute the lyophilized capture antibody, centrifuge at 10,000 g for 2 minutes. Add 660 µL of Sample Diluent NS, let sit at room temperature for 10 minutes and resuspend well by inverting the tube by hand and gently pipetting. Unused reconstituted antibody can be stored frozen at -20°C. Avoid repeated freeze-thaw cycles.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. 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 5BI. 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-6 standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 2,000 pg/mL **Stock Standard** Solution.
- 2. Label nine tubes, Standards 1–9.
- 3. Add 75 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS 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	225	75	2,000	1,500
2	Standard#1	75	150	1,500	500
3	Standard#2	75	150	500	166.67
4	Standard#3	75	150	166.67	55.56
5	Standard#4	75	150	55.56	18.52
6	Standard#5	75	150	18.52	6.17
7	Standard#6	75	150	6.17	2.06
8	Standard#7	75	150	2.06	0.69
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	≤50%
Plasma – Citrate	≤25%
Plasma – EDTA	≤50%
Stimulated PBMC Supernatant	1:3,200 - 1:200
Urine	≤50%
Cerebrospinal Fluid	≤50%

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 NS and assay. Store un-diluted serum at -20°C 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 plasma (citrate) samples at least 1:4 and plasma (EDTA) samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C 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 and dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Cerebrospinal Fluid (CSF) Dilute cerebrospinal fluid at least 1:2 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.

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

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human IL-6.

The standard protein in this kit is mature full length human IL-6.

Native signal was detected in cell culture supernatant and serum from one individual Rheumatoid arthritis donor.

Native signal was detected in three out of ten individual healthy serum samples.

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

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

50% cerebrospinal fluid pooled from 6 healthy human donors was measured in duplicate. All values were below the detectable range of the assay.

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

This kit is incompatible with plasma (heparin) samples.

CROSS REACTIVITY

50 ng/mL and 500 pg/mL of recombinant Human IL-6Ra was tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE

50 ng/mL and 500 pg/mL of recombinant Human IL-6Ra tested for interference with 62.5 pg/mL of recombinant Human IL-6. No interference was observed.

SPECIES REACTIVITY

500 pg/mL of recombinant Mouse IL-6 was tested for reactivity. No reactivity was observed.

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	1,990	1,891	1,941
0.69	3,241	3,592	3,417
2.06	4,881	5,260	5,071
6.17	11,809	13,971	12,890
18.5	27,951	30,722	29,337
55.6	95,327	102,260	98,794
166.7	252,110	269,700	260,905
500	711,810	742,770	727,290
1,500	2,097,100	2,112,700	2,104,900

Table 1. Example of human IL-6 standard curve in Sample Diluent NS. The IL-6 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 0.33 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=8) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of IL-6 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	82	77 - 84
25 % Plasma – Citrate	84	81 - 85
50% Plasma – EDTA	82	80 - 85
50% Cell Culture Media*	100	97 - 103
50% Urine	98	94 - 106
50% Cerebrospinal Fluid	90	87 - 93

*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.

Native IL-6 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	1:200 Stimulated PBMC Supernatant
Undiluted	pg/mL	311.1
	% Expected value	100
2	pg/mL	155.9
	% Expected value	100
4	pg/mL	75.8
	% Expected value	97
8	pg/mL	36.4
	% Expected value	94
16	pg/mL	18.3
	% Expected value	94

Recombinant IL-6 was spiked into 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 (EDTA)	50% Human CSF	50% Human Urine
Undiluted	pg/mL	199.5	108.2	207.7	242.4	418.3
	% Expected value	100	100	100	100	100
2	pg/mL	108.6	60.8	110.6	124.9	237.4
	% Expected value	109	112	106	103	114
4	pg/mL	55.6	34.2	58.1	64.3	113.6
	% Expected value	112	126	112	106	109
8	pg/mL	28.5	15.5	28.8	32.5	60.3
	% Expected value	114	115	111	107	115
16	pg/mL	14.8	NL	15.0	15.8	NL
	% Expected value	118	NL	115	104	NL

NL – Non-Linear

Precision

Mean coefficient of variations of interpolated values of IL-6 from a single concentration of stimulated PMBC supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	2.1	2.4

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

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