ab229441 – Human IL-2 CatchPoint® SimpleStep ELISA® Kit

For the quantitative measurement of IL-2 in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab229441

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 (ab270883).

Materials Supplied

Item	Quantity	Storage Condition
Human IL-2 Capture Antibody 10X	600 µL	+4°C
Human IL-2 Detector Antibody 10X	600 µL	+4°C
Human IL-2 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Sample Diluent 50BP	20 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
Stoplight Red Substrate Buffer	12 mL	+4°C
100X Stoplight Red Substrate	120 µL	+4°C
500X Hydrogen Peroxide (H_2O_2, 3%)	50 µL	+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:

Fluorescence microplate reader Ex/Cutoff/Em 530/570/590 nm.
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 50BP 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 4BI. 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 4BI. Mix thoroughly and gently.

CatchPoint HRP Development Solution: Just prior to use prepare CatchPoint HRP Development Solution by diluting the 100X Stoplight Red Substrate and the 500X Hydrogen Peroxide in Stoplight Red Substrate Buffer. For example, to make 6 mL of the CatchPoint HRP Development Solution combine 60 µL 100X Stoplight Red Substrate and 12 µL of 500X Hydrogen Peroxide with 5.928 mL Stoplight Red Substrate Buffer. Mix thoroughly and gently.

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-2** protein standard by adding the volume indicated on the protein vial label. For **serum**, **plasma (EDTA)**, **plasma (citrate)**, and **plasma (heparin)samples measurements**, use Sample Diluent 50BP. 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 40,000 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 300 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**	100	300	40,000	10,000
2	Standard#1	150	150	10,000	5,000
3	Standard#2	150	150	5,000	2,500
4	Standard#3	150	150	2,500	1,250
5	Standard#4	150	150	1,250	625
6	Standard#5	150	150	625	312.5

7	Standard#6	150	150	312.5	156.25
8	Standard#7	150	150	156.25	78.13
9	Standard#8	150	150	78.13	39.06
10	Standard#9	150	150	39.06	19.53
11	Blank Control	0	150	N/A	N/A

To convert sample values obtained with the kit to approximate NIBSC (86/500) units, use the following equation: NIBSC (86/500) approximate value (pg/mL) = 1.4 x SimpleStep Human IL-2 value (pg/mL).

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	≤ 25%
Plasma – EDTA	≤ 25%
Plasma – Citrate	≤ 25%
Plasma – Heparin	≤ 25%
Stimulated PBMCs	0.8 - 25%
RPMI 10% FBS Cell culture media	≤ 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 1:4 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, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:4 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.
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples 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 1 hour at room temperature on a plate shaker set to 400 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 10 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 100 µL of prepared CatchPoint HRP Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. Further optimization of incubation time vs signal strength can be performed if needed.
8. Record the fluorescence at Ex/Cutoff/Em 530/570/590 nm. If using a Molecular Devices' plate reader supported by SoftMax® Pro software, a preconfigured protocol for these CatchPoint SimpleStep ELISA Kits is available with all the protocol and analysis settings at www.softmaxpro.org.
9. Analyze the data as described below.

Mode	Fluorescence
Instrument settings:	Endpoint
Excitation:	530 nm
Cutoff:	570 nm
Emission:	590 nm
Sensitivity:	6 flashes/read or 200ms
PMT:	Auto
Auto calibrate:	On
Read:	Top
Read Height:	1*

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

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

All data, except Typical Standard Curve and Sensitivity were collected using the colorimetric version of this kit (ab270883).

ASSAY SPECIFICITY

This kit is designed for the quantification of human IL-2.

The standard protein in this kit is full length human IL-2.

Native signal was detected in cell culture supernatant.

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

25% pooled serum and plasma (EDTA, Heparin, Citrate) samples from healthy donors was measured in duplicate. All values were below the detectable range of the assay.

25% serum from eight individual healthy human female/male 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.

CROSS REACTIVITY

5,000 pg/mL of recombinant human IL-2R was tested for cross reactivity. No cross reactivity was observed.

SPECIES REACTIVITY

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices
- Calculate the average fluorescence value for the blank control (zero) standards. Subtract the average blank control standard fluorescence value from all other fluorescence values.
- Create a standard curve by plotting the average blank control subtracted fluorescence 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 fluorescence 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 fluorescence 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 fluorescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at fluorescence 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)	RFU		Mean RFU
	1	2	
0	1046741	980919	1013830
19.53	1943848	1478433	1711141
39.06	2250131	2115857	2182994
78.13	3307732	3101895	3204813
156.25	5105991	5439592	5272792
312.5	15830276	12840270	14335273
625	22517474	21521064	22019269
1,250	42029448	41590956	41810202
2,500	89732264	93886280	91809272
5,000	178994288	177442736	178218512
10,000	273130560	248643728	260887144

Table 1. Example of human IL-2 standard curve in Sample Diluent NS. The IL-2 standard curve was prepared as described in the Standard Preparation section. Raw data generated on SpectraMax M4 Multi-Mode Microplate Reader is shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

TYPICAL SAMPLE VALUES

Sensitivity:

The calculated minimal detectable dose (MDD) is 10.0 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=22) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of IL-2 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	86	83 - 88
25% Plasma - EDTA	89	86 - 93
25% Plasma - Citrate	87	84 - 91
25% Plasma - Heparin	82	80 - 85
5% Stimulated PBMC supernatant*	108	100 - 120
50% RPMI 10% FBS	96	91 - 103

*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-2 was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 50BS.

Dilution Factor	Interpolated value	25% Human Serum	25% Human Plasma (EDTA)	25% Human Plasma (Citrate)	25% Human Plasma (Heparin)
Undiluted	pg/mL	1,376	1,507	1,259	864
	% Expected value	100	100	100	100
2	pg/mL	672	716	591	499
	% Expected value	98	95	94	116
4	pg/mL	352	374	373	251
	% Expected value	102	99	119	116
8	pg/mL	165	190	190	137
	% Expected value	96	101	118	117
16	pg/mL	NL	106	NL	NL
	% Expected value		113		

NL – Non-Linear

Recombinant IL-2 was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human RPMI 10% FBS
Undiluted	pg/mL	1,242
	% Expected value	100
2	pg/mL	540
	% Expected value	87
4	pg/mL	278
	% Expected value	89
8	pg/mL	147
	% Expected value	95
16	pg/mL	68
	% Expected value	88

Native IL-2 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	25% PBMC
Undiluted	pg/mL	1,418
	% Expected value	100
2	pg/mL	648
	% Expected value	91
4	pg/mL	316
	% Expected value	89
8	pg/mL	168
	% Expected value	95
16	pg/mL	84
	% Expected value	95

Precision

Mean coefficient of variations of interpolated values of IL-2 from three concentrations of stimulated PBMC supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	4
CV (%)	3.2	8.7

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

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