ab190805 – Human CXCL1 SimpleStep ELISA® Kit (GRO alpha)

For the quantitative measurement of CXCL1 in human serum, plasma (heparin), plasma (citrate), cell culture supernatant, and CSF.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab190805

Storage and Stability: Store kit at 2-8ºC 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.

Materials Supplied

Item	Quantity 1x96 tests	Quantity 10x96 tests	Storage Condition
CXCL1 CXCL1 Capture Antibody 10X	600 µL	10 x 600 µL	+4ºC
CXCL1 CXCL1 Detector Antibody 10X	600 µL	10 x 600 µL	+4ºC
CXCL1 CXCL1 Lyophilized Recombinant Protein	2 Vials	10 x 2 vials	+4ºC
Antibody Diluent	6 mL	10 x 6 mL	+4ºC
Wash Buffer PT 10X	20 mL	200 mL	+4ºC
TMB Development Solution	12 mL	120 mL	+4ºC
Stop Solution	12 mL	120 mL	+4ºC
Sample Diluent NS	50 mL	2 x 250 mL	+4ºC
Anti-tag coated microplate (12 x 8 well strips)	96 Wells	10 x 96 wells	+4ºC
Plate Seal	1	10	+4ºC

Materials Required, Not Supplied

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

Microplate reader capable of measuring absorbance at 450 or 600 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

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 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.

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 CXCL1 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 4,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 385 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS 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**	15	385	4,000	150
2	Standard#1	150	150	150	75
3	Standard#2	150	150	75	37.5
4	Standard#3	150	150	37.5	18.75
5	Standard#4	150	150	18.75	9.38
6	Standard#5	150	150	9.38	4.69
7	Standard#6	150	150	4.69	2.34
8	Blank Control	0	150	N/A	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	12.5 - 100%
Platelet Poor Plasma - Heparin	12.5 - 100%
Platelet Poor Plasma - Citrate	25 - 100%
1.5% PHA-M Stimulated PBMC Cell Culture Supernatant	1:12,800 - 1:200
Unstimulated PBMC Cell Culture Supernatant	25 - 100%
Cerebrospinal Fluid*	≤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. Dilute samples as needed into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Platelet Poor Plasma Collect plasma on ice using sodium citrate or heparin as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Dilute samples as needed 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 - EDTA samples.

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 as needed into Sample Diluent NS and assay. Store un-diluted 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 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 TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

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 CXCL1.

The standard protein in this kit is mature full length human CXCL1.

Native signal was detected in serum, platelet poor plasma (heparin), platelet poor plasma (citrate), cell culture supernatant, and CSF.

Spiked protein experiments were used to validate CSF sample types.

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

This kit is incompatible with plasma (EDTA) samples.

CROSS REACTIVITY
CXCL3 was prepared at 200 pg/mL in Sample Diluent NS and assayed for cross-reactivity. 10% cross-reactivity was observed.

SPECIES REACTIVITY
Other species reactivity was determined by measuring 100% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Serum reactivity < 3% was determined for the following species: Mouse, Rat

No signal was observed from 100% cerebrospinal fluid samples from the following species: Monkey

Other species reactivity not determined.

CALCULATION
- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance 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.

  Δ Note: Most microplate 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 absorbance 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 absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance 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)	O.D 450 nm		Mean O.D
	1	2	
0	0.079	0.071	0.075
2.34	0.187	0.159	0.173
4.69	0.255	0.250	0.252
9.38	0.421	0.416	0.418
18.75	0.740	0.709	0.724
37.5	1.482	1.457	1.470
75	2.603	2.562	2.582
150	3.675	3.648	3.662

Table 1. Example of human CXCL1 standard curve in Sample Diluent NS. The CXCL1 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 0.064 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery
Three concentrations of CXCL1 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 (%)
12.5% Serum	83	82 - 85
25% Platelet Poor Plasma - Citrate	81	80 - 82
12.5% Platelet Poor Plasma - Heparin	86	85 - 87
25% PBMC Cell Culture Supernatant	95	92 - 97
50% CSF	96	93 - 101

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 CXCL1 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	100% Human Serum	100% Human Plasma (Citrate)	100% Human Plasma (Heparin)	50% Human Cerebrospinal Fluid	1:200 Stimulated PBMC Supernatant
Undiluted	pg/mL	18.78	10.58	27.06	2.23	94.88
	% Expected value	100	100	100	100	100
2	pg/mL	10.55	5.60	14.72	ND	40.78
	% Expected value	112	106	109	ND	86
4	pg/mL	5.51	3.00	7.78	ND	20.95
	% Expected value	117	114	106	ND	88
8	pg/mL	2.71	NL	4.00	ND	11.59
	% Expected value	116	NL	118	ND	98
16	pg/mL	ND	ND	ND	ND	5.71
	% Expected value	ND	ND	ND	ND	96

NL – Non-Linear
ND – Not Detected – below product dynamic range

Recombinant CXCL1 was spiked into the following human sample and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Cerebrospinal Fluid
Undiluted	pg/mL	41.48
	% Expected value	100
2	pg/mL	21.51
	% Expected value	104
4	pg/mL	11.38
	% Expected value	110
8	pg/mL	5.48
	% Expected value	106

Precision

Mean coefficient of variations of interpolated values of CXCL1 from a single concentration of PBMC supernatant treated with 1.5% PHA-M for 46 hours within the working range of the assay.

	Intra-assay	Inter-assay
N=	3	8
CV (%)	1.9	3.7

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

Technical Support

For all technical or commercial enquiries please go to:
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