Version 3a Last updated 3 October 2023

ab216951
Mouse CXCL1 ELISA Kit

For the quantitative measurement of mouse CXCL1 in serum, plasma, cell culture supernatant, and cell extract samples.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

CXCL1 *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of mouse CXCL1 protein in serum, plasma, cell culture supernatant, and cell extract samples.

The ELISA employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody. The capture antibody is incubated with the sample and then after washing, the detector antibody is added to the wells. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

CXCL1 is a member of the CXC family of chemokines. Chemokines play roles in normal and pathological processes including allergic responses, angiogenesis, inflammation, tumor growth and metastasis. Mouse CXCL2 and CXCL3 share 67% and 60% sequence homology with mouse CXCL1, respectively. Additionally, Rat CXCL1 is 89% homologous with mouse CXCL1.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Capture Antibody Cocktail to all wells and incubate at room temperature for 30 minutes

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL Detector Antibody Cocktail to all wells and incubate at room temperature for 30 minutes.

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Development Solution to each well and incubate for 10 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Mouse CXCL1 Capture Antibody 10X	600 µL	+4°C
Mouse CXCL1 Detector Antibody 10X	600 µL	+4°C
Mouse CXCL1 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	2 x 6 mL	+4°C
Wash Buffer PT 10X	2 x 20 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Anti-tag coated microplate (12 x 8 well strips)	96 Wells	+4°C
Plate Seal	1	+4°C

Note: Antibody Diluent CPI2- This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data equivalent to the original formulation of antibody diluent CPI previously used in this kit.
While we run stock down, you may receive kits containing antibody diluent CPI. This does not affect the way you should use the kit.
If you have any questions please contact Abcam Scientific Support.

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- 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).

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- 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.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. 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.

9.1 1X Cell Extraction Buffer PTR (For cell extracts only):

Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 110 mL 1X Wash Buffer PT combine 11 mL Wash Buffer PT 10X with 99 mL deionized water. Mix thoroughly and gently.

9.3 Capture Antibody:

Prepare Capture Antibody by diluting the 10X Capture Antibody in Sample Diluent NS and Antibody Diluent CPI2. To make 3 mL of the Capture Antibody combine 300 µL 10X Capture Antibody and 300 µL Sample Diluent NS with 2.4 mL Antibody Diluent CPI2. Mix thoroughly and gently.

9.4 Detector Antibody:

Prepare Detector Antibody by diluting the 10X Detector Antibody in Sample Diluent NS and Antibody Diluent CPI2. To make 6 mL of the Detector Antibody Cocktail combine 3.3 mL Sample Diluent NS and 300 µL 10X Detector Antibody into 2.4 mL Antibody Diluent CPI2. Mix thoroughly and gently.

10. Standard Preparation

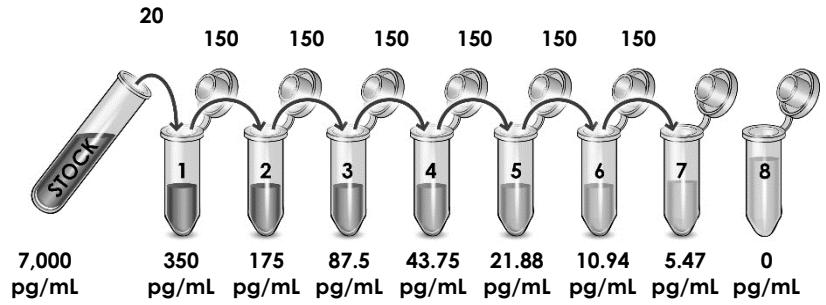
- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT**: If the protein standard vial has a volume identified on the label, reconstitute the mouse CXCL1 protein standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the mouse CXCL1 protein standard by adding 1,000 µL Diluent.
For **serum, plasma and cell culture supernatant sample measurements**, reconstitute the mouse CXCL1 protein standard by adding Sample Diluent NS.
For **cell extract sample measurements**, reconstitute the mouse CXCL1 protein standard by adding 1X Cell Extraction Buffer PTR.
Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 7,000 pg/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 380 µL of appropriate diluent (see step 10.1) into tube number 1 and 150 µL of appropriate diluent into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Plasma - Heparin	6.25 – 50%
Plasma - EDTA	6.25 – 50%
Plasma - Citrate	3.13 – 50%
Serum	6.25 – 50%
NIH 3T3 Cell Culture Supernatant	3.13 – 50%
NIH 3T3 Cell Extract	31.25 – 500 µg/mL

11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples 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.

11.2 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 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3 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.

11.4 Preparation of extracts from cell pellets:

11.4.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.4.2 Rinse cells twice with PBS.

11.4.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.4.4 Incubate on ice for 20 minutes.
11.4.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.
11.4.6 Transfer the supernatants into clean tubes and discard the pellets.
11.4.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.
11.4.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.5 Preparation of extracts from adherent cells by direct lysis (alternative protocol):

11.5.1 Remove growth media and rinse adherent cells 2 times in PBS.
11.5.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).
11.5.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.
11.5.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.
11.5.5 Transfer the supernatants into clean tubes and discard the pellets.
11.5.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.
11.5.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

12. 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.

13. 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.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Capture Antibody to each well.

13.5 Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 400 rpm.

13.6 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.

13.7 Add 100 µL of the Detector Antibody to each well.

13.8 Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 400 rpm.

13.9 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.

13.10 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.

13.11 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.

13.12 Alternative to 13.7 – 13.8: 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

Δ **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.

13.13 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **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.

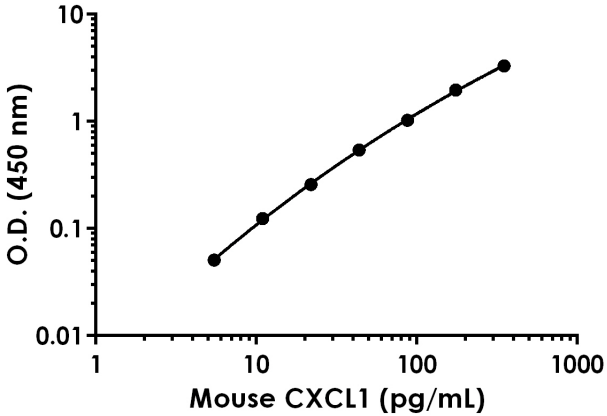
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 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.

14.4 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.

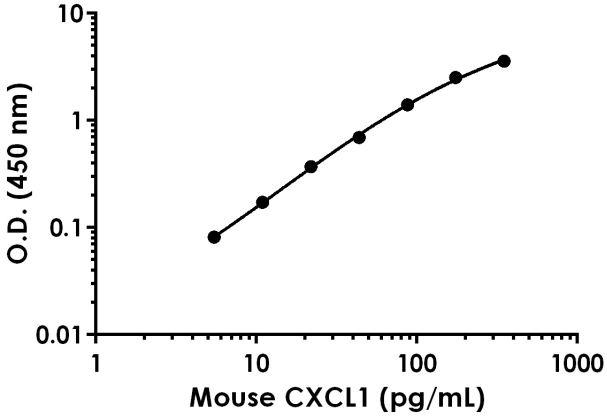
15. Typical Data

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	0.085	0.094	0.090
5.47	0.142	0.139	0.141
10.94	0.218	0.210	0.214
21.88	0.352	0.343	0.347
43.75	0.634	0.630	0.632
87.5	1.141	1.086	1.114
175	2.023	2.092	2.058
350	3.388	3.401	3.395

Figure 1. Example of mouse CXCL1 standard curve in Sample Diluent NS. The CXCL1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.076	0.085	0.081
5.47	0.165	0.159	0.162
10.94	0.252	0.254	0.253
21.88	0.456	0.447	0.451
43.75	0.795	0.751	0.773
87.5	1.495	1.472	1.484
175	2.604	2.600	2.602
350	3.661	3.670	3.665

Figure 2. Example of mouse CXCL1 standard curve in 1X Cell Extraction Buffer PTR. The CXCL1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The 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
1X Cell Extraction Buffer PTR	24	0.70 pg/mL
Sample Diluent NS	24	0.66 pg/mL

RECOVERY –

Three concentrations of mouse 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 (%)
Plasma – Heparin (50%)	79	72 – 90
Plasma – EDTA (50%)	94	89 – 98
Plasma – Citrate (50%)	94	92 – 97
Serum (50%)	91	88 – 94
Cell Culture Media (50%)	110	109 – 110
NIH 3T3 Cell Extract (200 µg/mL)	95	95 – 96

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 mouse CXCL1 was measured in NIH 3T3 cell culture supernatant and NIH 3T3 cell extract samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS for cell culture supernatant samples and sample dilutions are made in 1X Cell Extraction Buffer PTR for cell extract samples.

Recombinant mouse CXCL1 was spiked into mouse serum and plasma samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	50% NIH 3T3 Supernatant	500 µg/mL NIH 3T3 Cell Extract
Undiluted	pg/mL	264.83	92.23
	% Expected value	100	100
2	pg/mL	122.06	42.75
	% Expected value	92	93
4	pg/mL	58.09	22.14
	% Expected value	88	96
8	pg/mL	27.86	11.34
	% Expected value	84	98
16	pg/mL	13.62	5.51
	% Expected value	82	96

Dilution Factor	Interpolated value	50% Mouse Serum	50% Mouse Plasma (Citrate)	50% Mouse Plasma (EDTA)	50% Mouse Plasma (Heparin)
Undiluted	pg/mL	187.13	189.00	171.62	246.02
	% Expected value	100	100	100	100
2	pg/mL	87.22	90.33	84.43	121.54
	% Expected value	93	96	98	99
4	pg/mL	45.56	46.91	44.03	61.96
	% Expected value	97	99	103	101
8	pg/mL	28.39	26.99	26.04	34.29
	% Expected value	121	114	121	112
16	pg/mL	NL	14.03	NL	NL
	% Expected value	NL	119	NL	NL

NL – Non-Linear

PRECISION –

Mean coefficient of variations of interpolated values from mouse serum within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	8	3
CV(%)	6.8	10.1

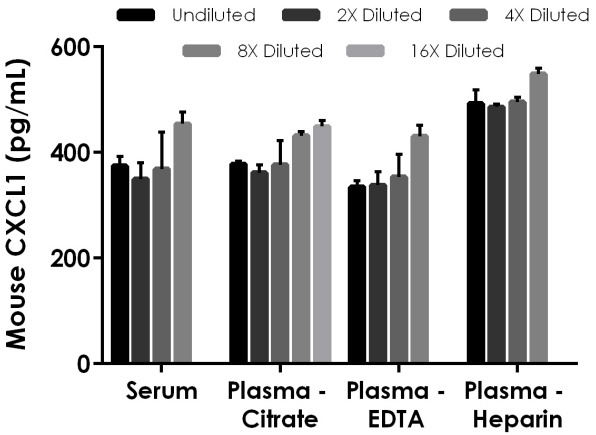


Figure 3. Interpolated concentrations of spiked CXCL1 in mouse serum and plasma samples. The concentrations of CXCL1 were measured in duplicates, interpolated from the CXCL1 standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 50%, plasma (citrate) 50%, plasma (EDTA) 50%, and plasma (heparin) 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CXCL1 concentration was determined to be 386.84 pg/mL in neat serum, 399.45 pg/mL in neat plasma (citrate), 364.34 pg/mL in neat plasma (EDTA), and 505.80 pg/mL in neat plasma (heparin).

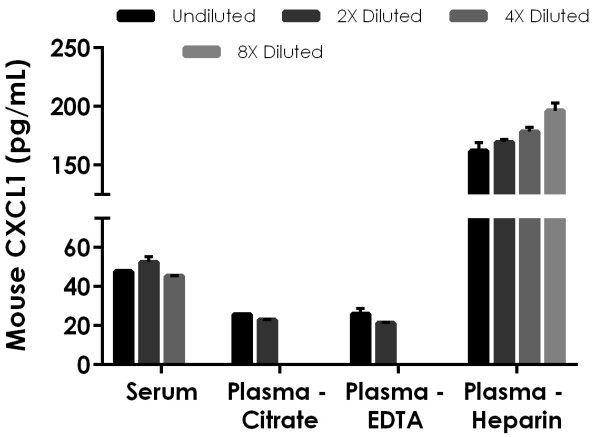


Figure 4. Interpolated concentrations of native CXCL1 in mouse serum and plasma samples. The concentrations of CXCL1 were measured in duplicates, interpolated from the CXCL1 standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 50%, plasma (citrate) 50%, plasma (EDTA) 50%, and plasma (heparin) 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CXCL1 concentration was determined to be 48.56 pg/mL in neat serum, 24.34 pg/mL in neat plasma (citrate), 23.67 pg/mL in neat plasma (EDTA), and 176.56 pg/mL in neat plasma (heparin).

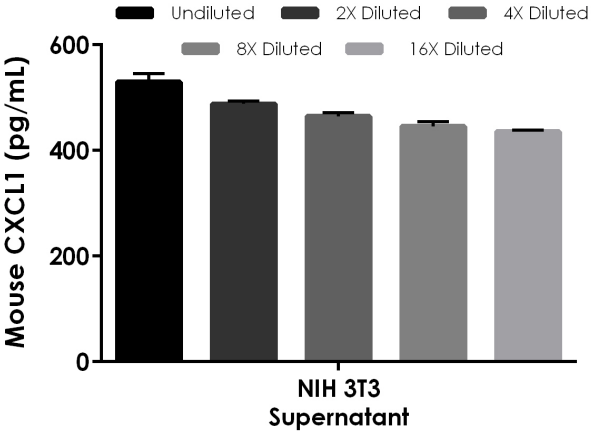


Figure 5. Interpolated concentrations of native CXCL1 in mouse NIH 3T3 cell culture supernatant sample. The concentrations of CXCL1 were measured in duplicates, interpolated from the CXCL1 standard curves and corrected for sample dilution. Undiluted samples are as follows: NIH 3T3 supernatant 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CXCL1 concentration was determined to be 472.88 pg/mL in neat mouse NIH 3T3 cell culture supernatant.

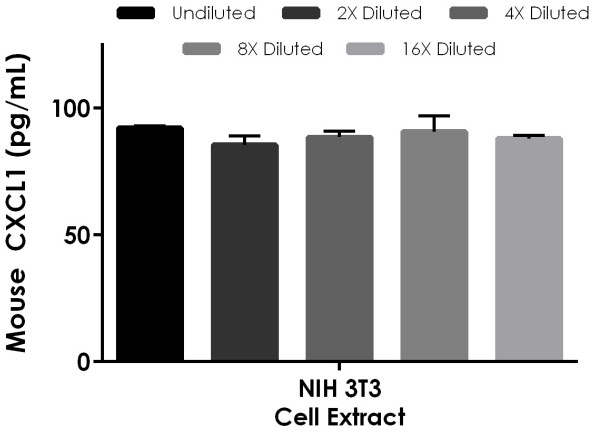


Figure 6. Interpolated concentrations of native CXCL1 in mouse NIH 3T3 cell extract sample based on a 500 µg/mL extract load. The concentrations of CXCL1 were measured in duplicate and interpolated from the CXCL1 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean CXCL1 concentration was determined to be 89.05 pg/mL in mouse NIH 3T3 cell extract sample.

17. Assay Specificity

This kit recognizes both native and recombinant mouse CXCL1 protein in serum, plasma, cell culture supernatant, and cell extract samples only.

CROSS REACTIVITY

Recombinant mouse CXCL2 and CXCL3 were prepared at 350 pg/mL each and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant mouse CXCL2 and CXCL3 were prepared at 350 pg/mL each and tested for interference. No interference was observed, with recovery of CXCL1 between 94 – 105%.

18. Species Reactivity

This kit recognizes mouse CXCL1 protein.

Other species reactivity was determined by measuring 50% serum samples of various species, interpolating the protein concentrations from the mouse standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in mouse serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Human
- Cow

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

20. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)