



# ab203364 – CXCL9 Mouse SimpleStep ELISA® Kit

## Instructions for Use

For the quantitative measurement of CXCL9 in mouse serum, plasma, cell culture supernatant, and cell and tissue extract samples.

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

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## 1. BACKGROUND

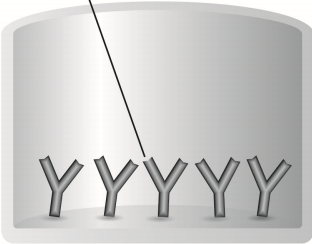
CXCL9 *in vitro* SimpleStep ELISA® kit is designed for the quantitative measurement of CXCL9 protein in mouse serum, plasma, cell culture supernatant, and cell and tissue extract samples.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. 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.

MIG (Monokine Induced by Gamma interferon) is a small cytokine belonging to the CXC motif chemokine family, thus named CXC motif chemokine family ligand 9. CXCL9 is a cytokine that affects the growth, movement, or activation state of cells that participate in immune and inflammatory response. CXCL9 is a T-cell chemoattractant, which is induced by IFN- $\gamma$ .

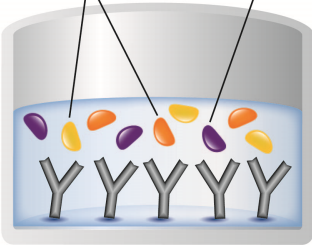
## 2. ASSAY SUMMARY

Immobilization Antibody



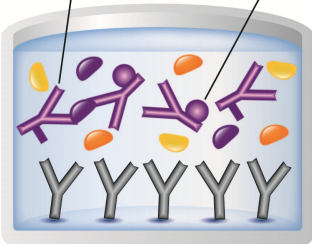
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.

Matrix Proteins Target Analyte



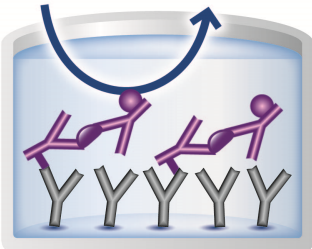
Add standard or sample to appropriate wells.

Capture Antibody Detector Antibody



Add Antibody Cocktail to all wells. Incubate at room temperature.

Substrate Color Development



Aspirate and wash each well. Add TMB Development Solution to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.

### 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. Modifications to the kit components or procedures may result in loss of performance.

### 4. 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 Reagent and Standard Preparation sections.

### 5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X Mouse CXCL9 Capture Antibody	600 µL	+2-8°C
10X Mouse CXCL9 Detector Antibody	600 µL	+2-8°C
Mouse CXCL9 Lyophilized Recombinant Protein	2 Vials	+2-8°C
Antibody Diluent CPI	6 mL	+2-8°C
10X Wash Buffer PT	20 mL	+2-8°C
5X Cell Extraction Buffer PTR	10 mL	+2-8°C
50X Cell Extraction Enhancer Solution	1 mL	+2-8°C
TMB Development Solution	12 mL	+2-8°C
Stop Solution	12 mL	+2-8°C
Sample Diluent NS	50 mL	+2-8°C
Sample Diluent 25BS	20 mL	+2-8°C
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8°C
Plate Seal	1	+2-8°C

## 6. 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.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH<sub>2</sub>PO<sub>4</sub>, 8 mM Na<sub>2</sub>HPO<sub>4</sub>, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

## 7. 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.

## 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 50X Cell Extraction Enhancer Solution may precipitate when stored at +4°C. To dissolve, warm briefly at +37°C and mix gently. The 50X Cell Extraction Enhancer Solution 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 Scientific 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 and tissue extracts only)

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL 5X Cell Extraction Buffer PTR. Mix thoroughly and gently. If required protease inhibitors can be added.

### 9.2 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

### 9.3 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI. To make 3 mL of the Antibody Cocktail combine 300  $\mu$ L 10X Capture Antibody and 300  $\mu$ L 10X Detector Antibody with 2.4 mL Antibody Diluent CPI. Mix thoroughly and gently.



## 10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following table describes the preparation of a standard curve for duplicate measurements (recommended).

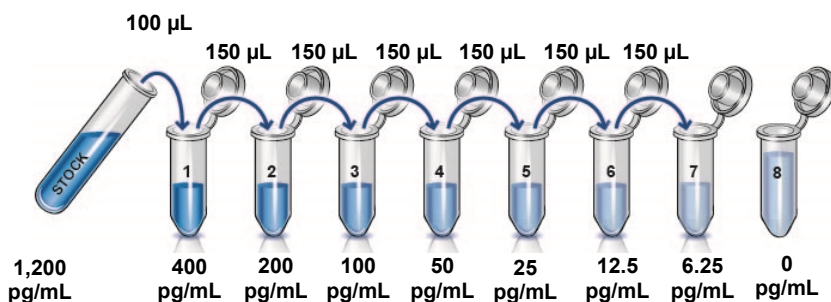
10.1 For **cell culture supernatant samples** follow this section.

**IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the CXCL9 standard by adding that volume of water indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the CXCL9 standard by adding 200  $\mu\text{L}$  water. Hold at room temperature for 10 minutes and mix gently. This is the 1,200  $\text{pg/mL}$  **Stock Standard** Solution.

10.1.1 Label eight tubes, Standards 1– 8.

10.1.2 Add 200  $\mu\text{L}$  Sample Diluent NS into tube number 1 and 150  $\mu\text{L}$  of Sample Diluent NS into numbers 2-8.

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



## ASSAY PREPARATION

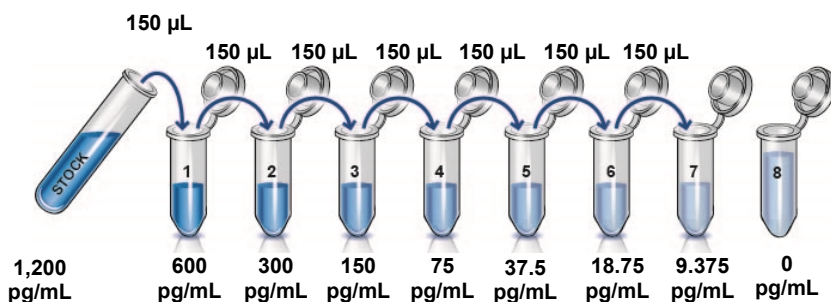
10.2 For **serum and plasma samples** follow this section.

**IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the CXCL9 standard by adding that volume of Sample Diluent 25BS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the CXCL9 standard by adding 200  $\mu\text{L}$  Sample Diluent 25BS. Hold at room temperature for 10 minutes and mix gently. This is the 1,200  $\text{pg/mL}$  **Stock Standard** Solution.

10.2.1 Label eight tubes, Standards 1– 8.

10.2.2 Add 150  $\mu\text{L}$  Sample Diluent 25BS into tube number 1 and 150  $\mu\text{L}$  of Sample Diluent 25BS into numbers 2-8.

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



## ASSAY PREPARATION

For **cell and tissue extract samples** measurements follow this section to prepare the standard.

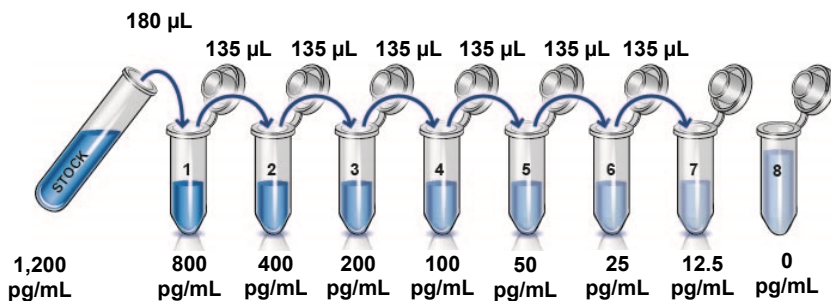
10.3 For **cell and tissue extract samples** follow this section.

**IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the CXCL9 standard by adding that volume of 1X Cell Extraction Buffer PTR indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the CXCL9 standard by adding 200  $\mu\text{L}$  1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix gently. This is the 1,200  $\text{pg/mL}$  **Stock Standard** Solution.

10.3.1 Label eight tubes, Standards 1– 8.

10.3.2 Add 90  $\mu\text{L}$  1X Cell Extraction Buffer PTR into tube number 1 and 135  $\mu\text{L}$  of 1X Cell Extraction Buffer PTR into numbers 2-8.

10.3.3 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
Mouse Serum	6.25 – 50 %
Mouse Plasma - Citrate	6.25 – 50 %
Mouse Plasma - EDTA	6.25 – 50 %
Mouse Plasma - Heparin	3.12 – 25 %
Mouse Liver Extract	31 – 500 µg/mL
Supernatant of Raw 264.7 cells treated for 72 hours with 500 ng/mL IFN-gamma	50X – 800X - diluted
Extract of Raw 264.7 cells treated for 72 hours with 500 ng/mL IFN-gamma	0.39 – 25 µg/mL

### 11.1 Plasma

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute plasma (citrate and EDTA) samples at least 2X into Sample Diluent 25BS and assay. Dilute plasma (heparin) samples at least 4X into Sample Diluent 25BS 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 serum samples at least 2X into Sample Diluent 25BS 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. Dilute supernatant samples at least 2X into Sample Diluent NS and assay. Store 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  $2 \times 10^7$  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  $\mu$ L - 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.
- 11.6 Preparation of extracts from tissue homogenates**
- 11.6.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).
  - 11.6.2 Homogenize 100 to 200 mg of wet tissue in 500  $\mu$ L – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.
  - 11.6.3 Incubate on ice for 20 minutes.
  - 11.6.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.
  - 11.6.5 Transfer the supernatants into clean tubes and discard the pellets.
  - 11.6.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.
  - 11.6.7 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.**
- **It is recommended to 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 4°C storage.
  - 13.3 Add 50 µL of all sample or standard to appropriate wells.
  - 13.4 Add 50 µL of the Antibody Cocktail to each well.
  - 13.5 Seal the plate and incubate for 1 hour 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. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
  - 13.7 Add 100 µL of TMB Substrate 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.*
  - 13.8 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.



## ASSAY PROCEDURE

*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

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

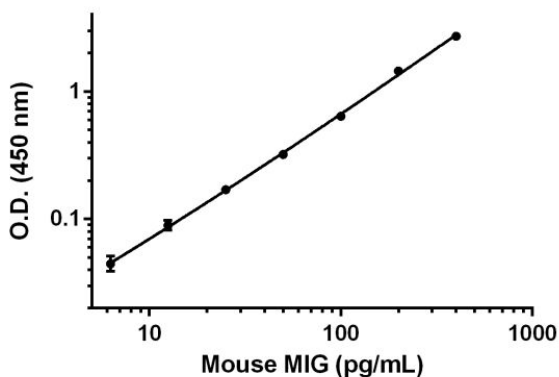
13.9 Analyze the data as described below.

## 14. CALCULATIONS

Subtract the average zero standard absorbance value from all measurements to determine the background-corrected absorbance value. Determine the average value of the duplicate background-subtracted measurements of the positive control dilutions and plot these values against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Software supplied with microplate readers have or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to determine the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). The concentrations of unknown samples can be interpolated from the curve using the background-corrected absorbance values and the dilution factor, if used. Absorbance values of samples which are greater than that of the highest standard should be diluted until the absorbance value falls within the linear part of the curve. The concentration of the sample can then be determined by applying the dilution factor.

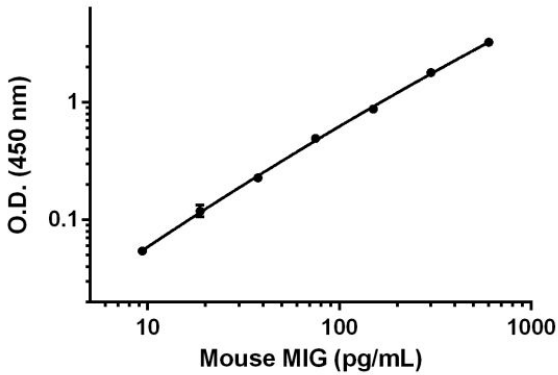
## 15. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



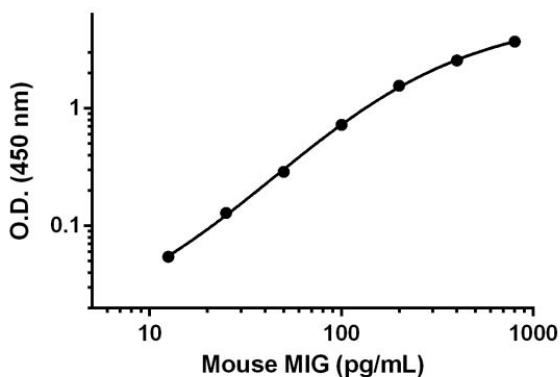
Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.087	0.069	0.079
6.25	0.127	0.118	0.123
12.5	0.173	0.162	0.168
25	0.255	0.242	0.249
50	0.403	0.397	0.401
100	0.721	0.718	0.720
200	1.523	1.543	1.533
400	2.778	2.828	2.804

**Figure 1.** Example of CXCL9 standard curve prepared in Sample Diluent NS. The CXCL9 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			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.056	0.056	0.056
9.375	0.113	0.108	0.111
18.75	0.185	0.166	0.176
37.5	0.291	0.279	0.286
75	0.581	0.525	0.553
150	0.912	0.958	0.936
300	1.818	1.888	1.854
600	3.287	3.343	3.315

**Figure 2.** Example of CXCL9 standard prepared in Sample Diluent 25BS. The CXCL9 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			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.0661	0.0657	0.066
12.5	0.1209	0.1203	0.121
25	0.1951	0.1958	0.195
50	0.3552	0.3560	0.356
100	0.7844	0.8025	0.793
200	1.7217	1.5416	1.632
400	2.632	2.6332	2.633
800	3.8015	3.741	3.771

**Figure 3.** Example of CXCL9 standard curve for prepared in 1X Cell Extraction Buffer PTR. The CXCL9 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 calculated minimal detectable dose (MDD) is determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentrations. The MDD is dependent on the Sample Diluent buffer used:

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	32	1.4 pg/mL
Sample Diluent 25BS	23	2.7 pg/mL
1X Cell Extraction Buffer PTR	18	6.3 pg/mL

### RECOVERY –

For **cell culture supernatant, serum and plasma samples** measurements, three concentrations of mouse recombinant CXCL9 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% Cell Culture Media	101.1	95.3 – 106
50% Mouse Serum	133.7	119.4 – 144
50% Mouse Plasma - Citrate	104.0	95.9 – 109.2
50% Mouse Plasma - EDTA	115.2	108.3 – 125.6
25% Mouse Plasma - Heparin	87.9	87.8 – 88.1

### 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.

## DATA ANALYSIS

Native mouse CXCL9 was measured in the following biological samples in a 2-fold dilution series. Cell Culture supernatant samples dilutions were made in Sample Diluent NS. Serum and plasma samples dilutions were made in Sample Diluent 25BS. Cell and tissue extract samples dilutions were made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	50% Mouse Serum	50% Mouse Plasma (Citrate)	50% Mouse Plasma (EDTA)	25% Mouse Plasma (Heparin)
Undiluted	pg/mL	208.05	158.48	294.45	166.95
	<b>% Expected value</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
2	pg/mL	124.67	81.02	148.03	85.67
	<b>% Expected value</b>	<b>119.8</b>	<b>102.3</b>	<b>100.6</b>	<b>102.6</b>
4	pg/mL	51.09	37.28	67.90	42.26
	<b>% Expected value</b>	<b>98.2</b>	<b>94.1</b>	<b>92.2</b>	<b>101.3</b>
8	pg/mL	24.64	19.17	30.26	22.05
	<b>% Expected value</b>	<b>94.8</b>	<b>96.8</b>	<b>82.2</b>	<b>105.6</b>
16	pg/mL	10.10	NA	NA	NA
	<b>% Expected value</b>	<b>77.6</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>

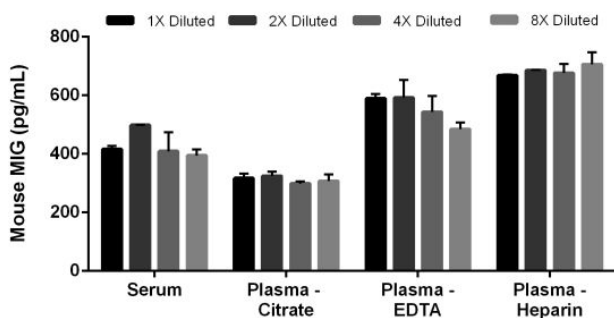
NA – Not Analyzed, measured O.D. values were lower than the O.D. value of the lowest standard

Dilution Factor	Interpolated value	25 µg/mL Raw 264.7 IFN $\gamma$ Extract	500 µg/mL Mouse Liver Extract	2% Raw 264.7 IFN $\gamma$ Supernatant
Undiluted	pg/mL	961.62	689.00	568.91
	<b>% Expected value</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>
2	pg/mL	509.79	324.46	294.7
	<b>% Expected value</b>	<b>106.0</b>	<b>94.2</b>	<b>103.6</b>
4	pg/mL	225.22	175.37	146.88
	<b>% Expected value</b>	<b>93.7</b>	<b>101.8</b>	<b>103.3</b>
8	pg/mL	103.16	91.73	81.61
	<b>% Expected value</b>	<b>85.8</b>	<b>106.5</b>	<b>114.8</b>
16	pg/mL	52.22	51.57	43.78
	<b>% Expected value</b>	<b>86.9</b>	<b>119.8</b>	<b>123.1</b>

## PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations supernatants of RAW 264.7 cells treated for 72 hours with mouse Recombinant IFN-gamma within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	5	3
CV (%)	7.6	7.5

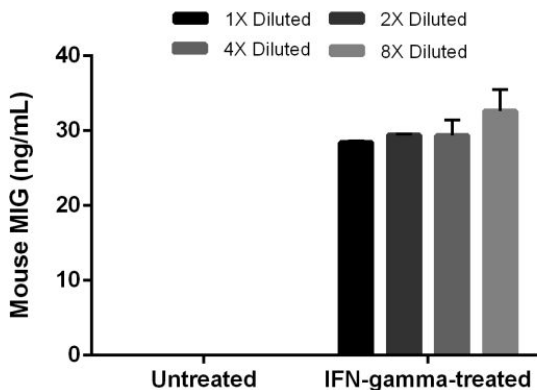


**Figure 4.** Interpolated concentrations of CXCL9 in mouse serum and plasma samples. The concentrations of CXCL9 were measured in duplicates, interpolated from the CXCL9 standard curve and corrected for sample dilution. Note that 1X Diluted serum and plasma (citrate and EDTA) samples were pre-diluted to 50%. Note that 1X Diluted plasma (heparin) samples were pre-diluted to 25%. The interpolated, dilution factor-corrected values are plotted in pg of CXCL9 per mL of neat sample (mean +/- SD, n=2).

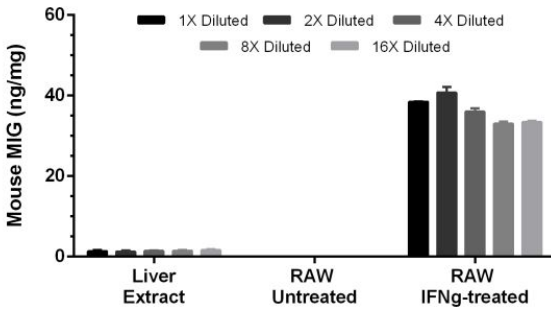


**Serum and Plasma.** Pooled female mouse serum and plasma samples were measured in three different dilutions in duplicates for CXCL9 concentrations using this kit. Means of interpolated sample values adjusted to sample dilution are shown in pg of CXCL9 per mL of serum/plasma in the table below.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Mouse Serum	441	409 - 499
Mouse Plasma - Citrate	313	298 - 324
Mouse Plasma - EDTA	575	543 - 592
Mouse Plasma - Heparin	676	668 - 685



**Figure 5.** IFN-gamma treatment of Raw 264.7 cell induces secretion of CXCL9. Raw 264.7 cells were cultured in the absence or presence of 500 ng/mL mouse recombinant Interferon-gamma protein for 72 hours. The cell culture supernatant samples were collected. The concentrations of CXCL9 were measured in the diluted supernatants in duplicates, interpolated from the CXCL9 standard curve and corrected for sample dilution. Note that 1X Diluted supernatant samples were pre-diluted to 2%. The interpolated, dilution factor-corrected values are plotted in ng of CXCL9 per mL of neat sample (mean  $\pm$  SD, n=2).



**Figure 6.** IFN-gamma treatment of Raw 264.7 cell induces expression of CXCL9. Raw 264.7 cells were cultured in the absence or presence of 500 ng/mL Mouse recombinant Interferon-gamma protein for 72 hours. The concentrations of CXCL9 were measured in diluted extracts of these cells and of Mouse liver in duplicates, interpolated from the CXCL9 standard curve and corrected for sample dilution. Note that 1X Diluted Raw 264.7 cells extract samples were at 25  $\mu\text{g/mL}$ . Note that 1X Diluted Mouse liver extract samples were at 500  $\mu\text{g/mL}$ . The interpolated, dilution factor-corrected values are plotted in ng of CXCL9 per mg of total protein (mean  $\pm$  SD, n=2).

### **17. ASSAY SPECIFICITY**

This kit recognizes both native and recombinant mouse CXCL9 protein in serum, plasma, cell culture supernatant, and cell and tissue extract samples only.

Urine, milk and saliva samples have not been tested with this kit.

### **CROSS REACTIVITY**

Recombinant mouse GRO-alpha (CXCL1) and MIP2 (CXCL2) proteins were tested at 400, 200, 100 and 50 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

## 18. SPECIES REACTIVITY

This kit recognizes mouse CXCL9 protein.

Other species reactivity was determined by measuring 2X diluted 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:

- Rabbit
- Dog
- Pig
- Cow
- Human

Please contact our Scientific Support team for more information.

## 19. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
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 Development 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**

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