ab204517 – MIP2 (CXCL2) Mouse SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of MIP2 (CXCL2) in mouse serum, plasma, and cell culture supernatants.

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

Version 2 Last Updated 21 June 2022

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1. BACKGROUND

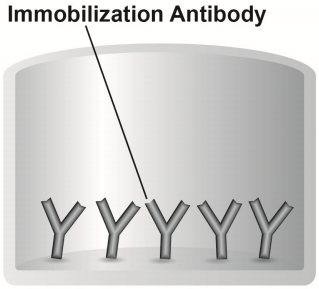
MIP2 (CXCL2) mouse *in vitro* SimpleStep ELISA® kit is designed for the quantitative measurement of MIP2 protein in mouse serum, plasma, and cell culture supernatants.

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 substrate 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.

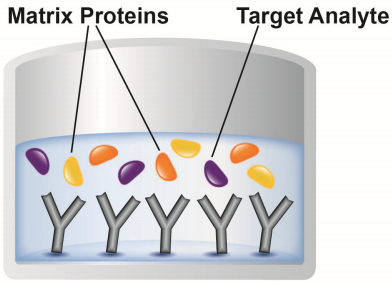
Mouse Macrophage Inflammatory Protein-2 (MIP2), also known as C-X-C motif chemokine 2 (CXCL2), is a small cytokine belonging to the CXC chemokine family. MIP2 was originally identified as a heparin-binding protein, and has been shown to exhibit potent neutrophil chemotactic activity. Mouse MIP2 c-DNA encodes a 100 amino acid residue precursor protein. The amino-terminal 27 amino acid residues are cleaved from this precursor to generate the mature mouse MIP2. Mouse MIP2 is 63% identical to Mouse KC (another mouse alpha chemokine), and mouse MIP2 is 60% identical to human GROβ and GROγ. Based on these protein sequence similarities, it is likely that mouse MIP2 and KC are homologs of the human GROα, β, and γ chemokines. However, since chemokines with protein sequence homology to human IL-8 have not been identified in mice, it has been suggested that the mouse MIP2 and KC are functional homologs of

human IL-8 in mice. A putative mouse homolog of the human IL-8 receptor beta (IL-8 Rβ) has also been cloned. This receptor shows 71% identity to human IL-8 Rβ and 68% identity to human IL-8 Rα. Both mouse MIP2 and KC bind mouse IL-8 Rβ with high affinity.

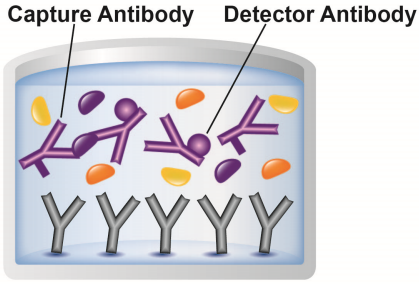
2. ASSAY SUMMARY



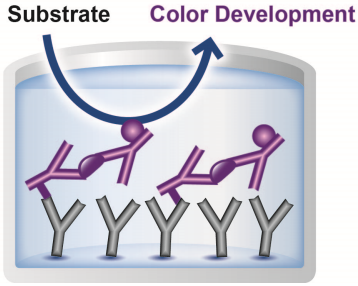
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



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



Aspirate and wash each well. Add TMB Substrate 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 2-8ºC 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 Reagent and Standard Preparation sections.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Mouse MIP2 Capture Antibody (Lyophilized)	1 vial	+2-8ºC
10X Mouse MIP2 Detector Antibody	600 µL	+2-8ºC
Mouse MIP2 Lyophilized Recombinant Protein	2 Vials	+2-8ºC
Antibody Diluent 4BI	6 mL	+2-8ºC
10X Wash Buffer PT	20 mL	+2-8ºC
TMB Substrate	12 mL	+2-8ºC
Stop Solution	12 mL	+2-8ºC
Sample Diluent NS	50 mL	+2-8ºC
Sample Diluent 50BP	20 mL	+2 - 8ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8ºC
Plate Seal	1	+2-8ºC

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 KH2PO4, 8 mM Na2HPO4, 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.
- **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 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.

9.2 10X Capture antibody

Prepare the 10X Capture antibody solution by adding 330 µL distilled H_2O and 330 µL Sample Diluent NS to the lyophilized capture antibody. Hold at room temperature for 10 minutes and mix thoroughly and gently

9.3 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.

9.4 Sample Diluent 25BP (For plasma citrate or EDTA samples)

Prepare Sample Diluent 25BP by diluting Sample Diluent 50BP in Sample Diluent NS. For example, to prepare 10 mL of Sample Diluent 25BP, mix 5 mL of Sample Diluent 50BP with 5 mL of Sample Diluent NS. 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).

IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the MIP2 standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the MIP2 standard by adding 1 mL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 2,000 pg/mL **Stock Standard** Solution.

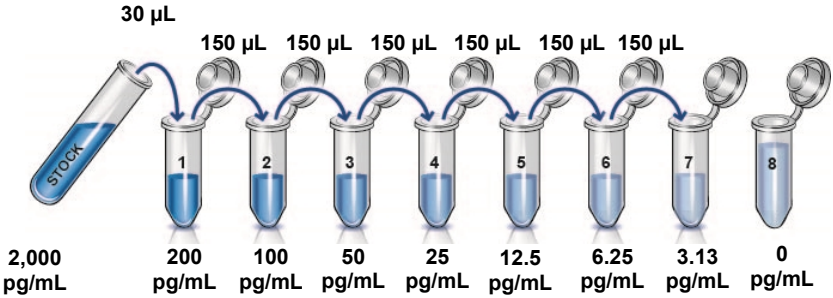
10.1 For **cell culture supernatant samples,** follow these instructions:

10.1.1 Reconstitute the MIP2 standard by of Sample Diluent NS by pipette.

10.1.2 Label eight tubes, Standards 1– 8.

10.1.3 Add 270 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.

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



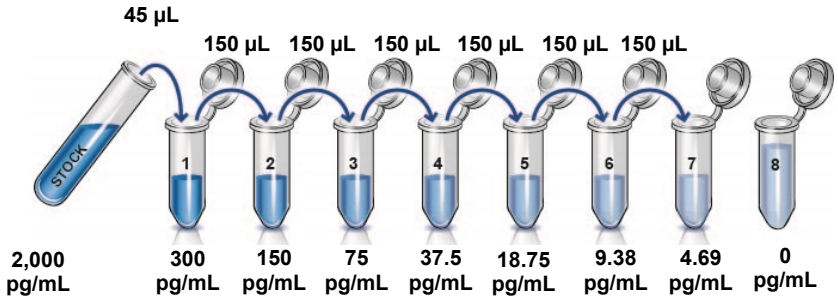
10.2 For **serum and heparin plasma samples**, follow these instructions:

10.2.1 Reconstitute the MIP2 standard by adding of Sample Diluent 50BP by pipette.

10.2.2 Label eight tubes, Standards 1– 8.

10.2.3 Add 255 µL of Sample Diluent 50BP into tube number 1 and 150 µL of Sample Diluent 50BP into numbers 2-8.

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



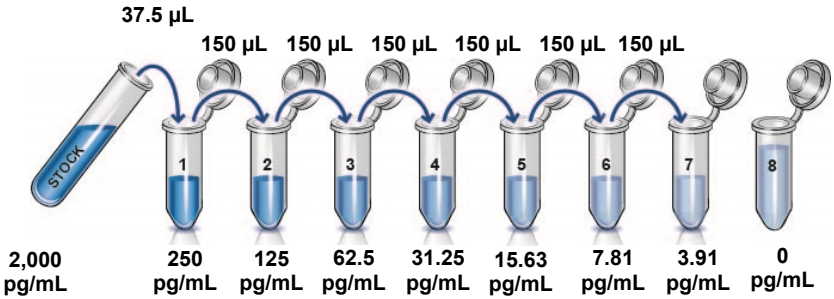
10.3 For **citrate and EDTA plasma samples** follow these instructions:

10.3.1 Reconstitute the MIP2 standard by adding Sample Diluent 25BP by pipette.

10.3.2 Label eight tubes, Standards 1– 8.

10.3.3 Add 262.5 µL of Sample Diluent 25BP into tube number 1 and 150 µL of Sample Diluent 25BP into numbers 2-8.

10.3.4 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**
Mouse Serum	1:1 – 1:16
Mouse Plasma - Citrate	1:1 – 1:16
Mouse Plasma - EDTA	1:2 – 1:32
Mouse Plasma - Heparin	1:1 – 1:16
Cell Culture Media	1:10 – 1:160
PHA+PMA stimulated J774A.1 cultured media	1:200 – 1:3,200
LPS stimulated RAW264.7 culture media	1:50 – 1:800
PHA stimulated RAW264.7 culture media	1:50 – 1:800

11.1 Plasma

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute citrate and EDTA samples into Sample Diluent 25BP and dilute heparin samples into Sample Diluent 50BP and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Sample Type	Average % Recovery	Range (%)
100% Mouse Serum	108	103 - 113
100% Mouse Plasma - Citrate	91	87 - 97
50% Mouse Plasma - EDTA	115	113 - 116
100% Mouse Plasma - Heparin	104	101 - 109
10% Cell Culture Media	102	98 - 107

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 50BP and assay. Store un-diluted serum at -20ºC 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.

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 4ºC 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 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.

13.8 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.

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 µL Stop Solution to each well and recording the OD at 450 nm.

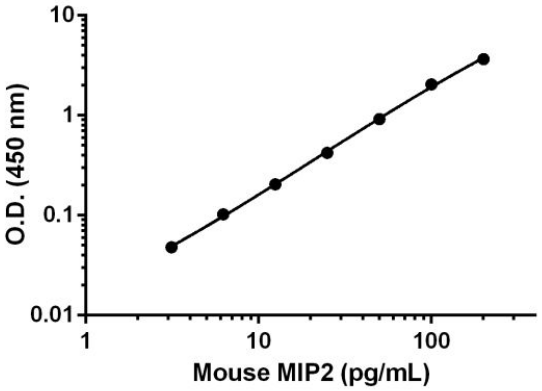
13.9 Analyze the data as described below.

14. CALCULATIONS

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software 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 see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

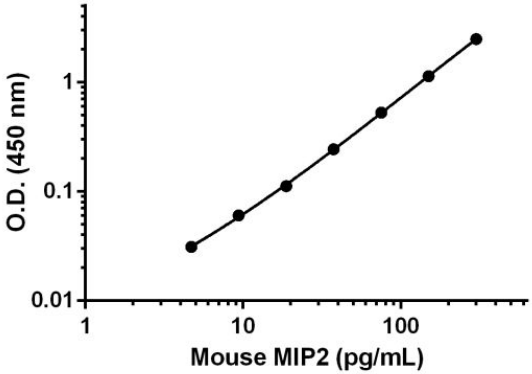
15. TYPICAL DATA

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



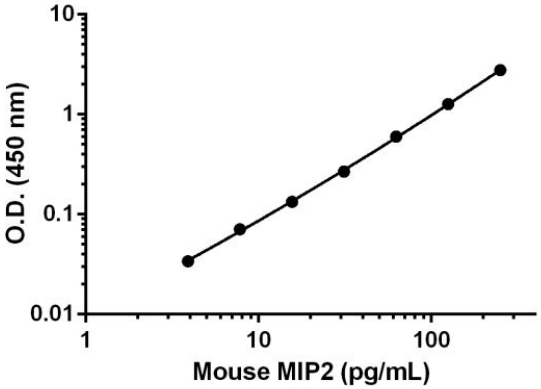
Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.053	0.054	0.053
3.13	0.103	0.100	0.101
6.25	0.154	0.157	0.156
12.5	0.266	0.251	0.259
25	0.464	0.488	0.476
50	0.950	0.991	0.971
100	2.058	2.133	2.096
200	3.699	3.744	3.721

Figure 1. Example of the mouse MIP2 standard curve in Sample Diluent NS. The mouse MIP2 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	0.063	0.065	0.064
4.69	0.096	0.095	0.095
9.38	0.125	0.124	0.124
18.75	0.179	0.173	0.176
37.5	0.313	0.301	0.307
75	0.604	0.582	0.593
150	1.187	1.212	1.199
300	2.552	2.543	2.547

Figure 2. Example of the mouse MIP2 standard curve in Sample Diluent 50BP. The mouse MIP2 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	0.059	0.060	0.059
3.91	0.096	0.091	0.093
7.81	0.131	0.129	0.130
15.63	0.190	0.195	0.193
31.25	0.318	0.336	0.327
62.5	0.649	0.674	0.662
125	1.307	1.345	1.326
250	2.844	2.823	2.833

Figure 3. Example of the mouse MIP2 standard curve in Sample Diluent 25BP. The Mouse MIP2 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	24	0.87 pg/mL
Sample Diluent 50BP	24	1.62 pg/mL
Sample Diluent 25BP	24	1.03 pg/mL

RECOVERY –

Three concentrations of recombinant mouse MIP2 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 (%)
100% Mouse Serum	108	103 - 113
100% Mouse Plasma - Citrate	91	87 - 97
50% Mouse Plasma - EDTA	115	113 - 116
100% Mouse Plasma - Heparin	104	101 - 109
10% Cell Culture Media	102	98 - 107

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 Mouse MIP2 was spiked into mouse serum and heparin plasma and diluted in a 2-fold dilution series in Sample Diluent 50BP. Recombinant mouse MIP2 was spiked into mouse citrate and EDTA plasmas and diluted in a 2-fold dilution series in Sample Diluent 25BP.

Dilution Factor	Interpolated value	100% Mouse Serum	100% Mouse Plasma (Citrate)	50% Mouse Plasma (EDTA)	100% Mouse Plasma (Heparin)
Undiluted	pg/mL	236.32	154.31	112.95	176.87
	% Expected value	**100**	**100**	**100**	**100**
2	pg/mL	121.53	76.87	52.78	98.57
	% Expected value	**103**	**100**	**93**	**111**
4	pg/mL	53.22	39.87	24.29	49.04
	% Expected value	**90**	**103**	**86**	**111**
8	pg/mL	24.81	20.54	11.95	23.95
	% Expected value	**84**	**106**	**85**	**108**
16	pg/mL	12.13	10.93	5.62	10.15
	% Expected value	**82**	**113**	**80**	**92**

Native Mouse MIP2 was measured in stimulated J774A.1 cultured media in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS. Recombinant mouse MIP2 was spiked into cell culture media and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	10% Cell Culture Media	0.5% J774A.1 Stimulated Media
Undiluted	pg/mL	73.50	194.90
	% Expected value	**100**	**100**
2	pg/mL	33.91	102.64
	% Expected value	**92**	**105**
4	pg/mL	19.14	48.26
	% Expected value	**104**	**99**
8	pg/mL	10.55	23.10
	% Expected value	**115**	**95**
16	pg/mL	4.16	10.11
	% Expected value	**90**	**83**

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of J774A.1 stimulated media within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	8	3
CV (%)	1.64	4.87

SAMPLE DATA –

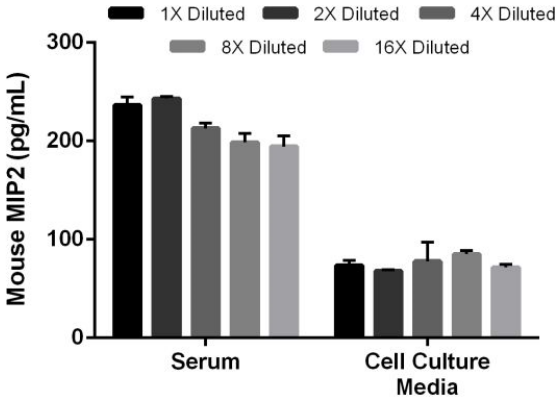


Figure 4. Linearity of dilution of MIP2 in serum and cell culture media. Recombinant mouse MIP2 was spiked into 100% serum and diluted in a 2-fold dilution series in Sample Diluent 50BP. Recombinant mouse MIP2 was spiked into 10% cell culture media and diluted in a 2-fold dilution series in Sample Diluent NS. The concentrations of mouse MIP2 were measured in duplicate and interpolated from the mouse MIP2 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD).

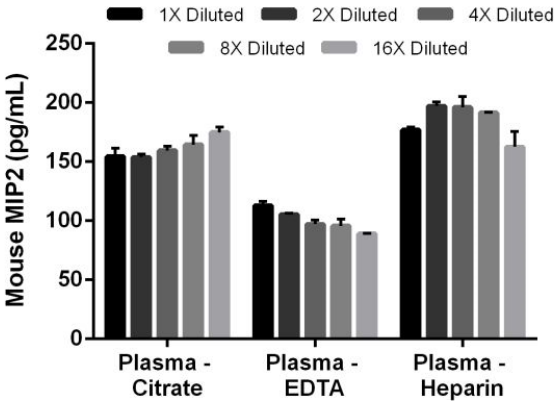


Figure 5. Linearity of dilution of spiked recombinant MIP2 in plasma. Recombinant mouse MIP2 was spiked into 100% citrate plasma and 50% EDTA plasma and diluted in a 2-fold dilution series in Sample Diluent 25BP. Recombinant Mouse MIP2 was spiked into 100% heparin plasma and diluted in a 2-fold dilution series in Sample Diluent 50BP. The concentrations of mouse MIP2 were measured in duplicate and interpolated from the mouse MIP2 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD).

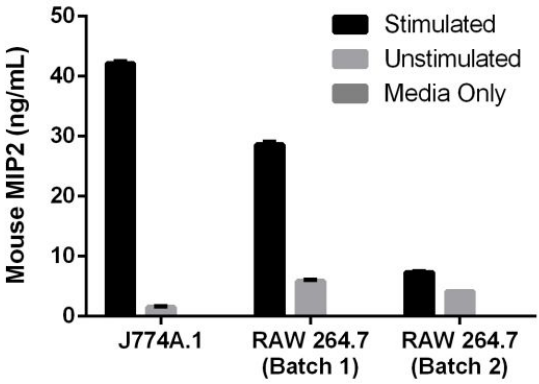


Figure 6. Mouse MIP2 expression is shown for cultured media from two mouse cell lines. J774A.1 cells were cultured in HGDMEM with 10% fetal calf serum, and 100 µg/mL of Kanamycin. During the exponential growth phase, J774A.1 cells were treated for 72 hours in the presence and absence of 1.5% PHA and 10 ng/mL of PMA. RAW 264.7 cells were cultured in HGDMEM with 10% fetal calf serum, 2 mM L-glutamine and 100 µg/mL Kanamycin. During the exponential growth phase, RAW264.7 cells were starved for 24 hours and treated in the presence and absence of 5 µg/mL of LPS (Batch #1) or 1% PHA (Batch #2).The concentrations of mouse MIP2 were interpolated from a standard curve diluted in Sample Diluent NS and corrected for sample dilution. The interpolated dilution factor corrected values are graphed (mean +/- SD).

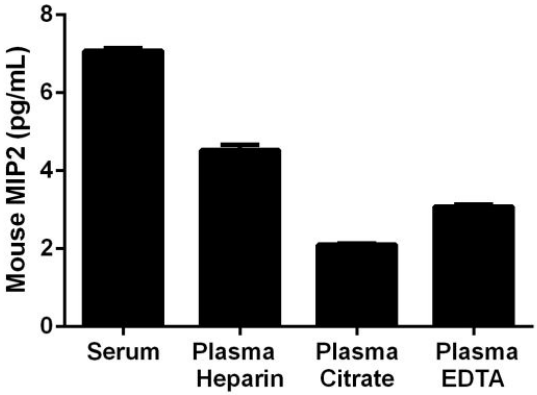


Figure 7. Mouse MIP2 expression is shown for serum and plasma samples. Native MIP2 was measured in duplicate in 100% serum and 100% heparin plasma and concentrations interpolated from a standard curve diluted in Sample Diluent 50BP. Native MIP2 was measured in duplicate in 100% Citrate plasma and 50% EDTA plasma and concentrations interpolated from a standard curve diluted in Sample Diluent 25BP. The interpolated dilution factor corrected values are graphed (mean +/- SD).

17. ASSAY SPECIFICITY

This kit recognizes both native and recombinant mouse MIP2 protein in serum, plasma, and cell culture supernatant samples only.

CROSS REACTIVITY

Mouse CXCL1, CXCL3, and CXCL4, were prepared at 5 ng/mL in Sample Diluent NS and assayed for cross-reactivity. No cross reactivity was observed for any of these samples, with a mean O.D. deviation from background of 0.0053.

INTERFERENCE

Recombinant mouse MIP2 was assayed at 75 pg/mL in the presence and absence of 5 ng/mL of Mouse CXCL1, CXCL3, and CXCL4. After background subtraction, recovery of mouse CXCL2 in the presence of mouse CXCL1, CXCL3, and CXCL4 was 104%, 103%, and 94%, respectively.

18. SPECIES REACTIVITY

This kit recognizes mouse MIP2 protein.

Human CXCL1, CXCL2, and CXCL3 were prepared at 5 ng/mL in Sample Diluent NS and assayed for cross-reactivity. No significant cross reactivity was observed for human CXCL1, CXCL2 or CXCL3 with a mean O.D. deviation from background of 0.0099.

Rat CXCL2 was prepared at 5 ng/mL in Sample Diluent NS and assayed for cross-reactivity. Reactivity for rat CXCL2 was determined at 3%.

Other species reactivity was determined by measuring 100% 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:

- Hamster
- Rabbit
- Dog

Please contact our Technical Support team for more information

19. TROUBLESHOOTING

Problem	Cause	Solution
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 37ºC.

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)