ab327291 – Mouse MIP2 SimpleStep ELISA® Kit (CXCL2), Chemiluminescent

For the quantitative measurement of MIP2 in mouse serum, plasma (heparin), plasma (EDTA), plasma (citrate), and cell culture supernatant.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab327291

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve and sensitivity were collected using the colorimetric version of this kit (ab204517).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Mouse MIP2 Capture (Lyophilized)	1 Vial	10 x 1 Vial	+4°C
Mouse MIP2 Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Mouse MIP2 Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4BI	6 mL	10 x 6 mL	+4°C
Sample Diluent 50BP	20 mL	10 x 20 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

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

Luminometer capable of reading strip plates with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent 50BP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

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

Sample Diluent 25BP: Prepare Sample Diluent 25BP by combining Sample Diluent NS and Sample Diluent 50BP. To make 5 mL Sample Diluent 25BP, combine 2.5 mL Sample Diluent NS and 2.5 ml Sample Diluent 50BP. Mix thoroughly and gently.

10X Capture Antibody: To reconstitute the lyophilized capture antibody, centrifuge at 10,000 g for 2 minutes. Add 330 µL of deionized water and 330 µL of Sample Diluent NS, let sit at room temperature for 10 minutes and resuspend well by inverting the tube by hand and gently pipetting. Unused reconstituted antibody can be stored frozen at -20°C. Avoid repeated freeze-thaw cycles.

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.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

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 (recommenIf ded).

Reconstitute the **MIP2** protein standard by adding the volume indicated on the protein vial label. For **Plasma EDTA and Plasma Citrate samples measurements**, use Sample Diluent 25BP. For **Cell Culture Supernatant samples measurements**, use Sample Diluent NS. For **Serum and Plasma Heparin samples measurements**, use Sample Diluent 50BP. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 2,000 pg/mL **Stock Standard** Solution.

For Plasma EDTA, Plasma Citrate, and cell culture supernatant samples use the instructions below:

1. Label nine tubes. Standards 1– 9.
2. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 150 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-9.
3. Use the **Stock Standard** to prepare the following dilution series. Standard #9 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**	150	150	2,000	1,000
2	Standard#1	75	150	1,000	333
3	Standard#2	75	150	333	111
4	Standard#3	75	150	111	37.0
5	Standard#4	75	150	37.0	12.4
6	Standard#5	75	150	12.4	4.12
7	Standard#6	75	150	4.12	1.37
8	Standard#7	75	150	1.37	0.46
9	Blank Control	0	150	0	0

For **Serum and Plasma Heparin samples measurements**, use the instructions below:

1. Label eight tubes, Standards 1– 8.
2. Add 150 µL of Sample Diluent 50BP into tube number 1 and 150 µL of Sample Diluent 50BP into numbers 2-8.
3. 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**	150	150	2,000	1,000
2	Standard#1	75	150	1,000	333
3	Standard#2	75	150	333.33	111
4	Standard#3	75	150	111.11	37.0
5	Standard#4	75	150	37.04	12.4
6	Standard#5	75	150	12.35	4.12
7	Standard#6	75	150	4.12	1.37
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	6.25% - 100%
Plasma – Citrate	6.25% - 100%
Plasma – EDTA	3.125% - 50%
Plasma – Heparin	6.25% - 100%
Cell Culture Media	0.625% - 10%
PHA+PMA stimulated J774A.1 Cell Culture Supernatant	1:3,200 - 1:200
LPS stimulated RAW264.7 Cell Culture Supernatant	1:800 – 1:50
PHA stimulated RAW264.7Cell Culture Supernatant	1:800 – 1:50

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Assay, or dilute samples into Sample Diluent 50BP and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.
Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Assay, or dilute citrate and EDTA samples into Sample Diluent 25BP and assay. Assay, or dilute heparin samples into Sample Diluent 50BP and assay. If required, dilute citrate and EDTA plasma samples into Sample Diluent 25BP and dilute heparin plasma samples into Sample Diluent 50BP. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples 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 30 minutes at room temperature on a plate shaker set to 750 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 30 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 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

Version 1 | 2026-05-04

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
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https://www.abcam.co.jp/contact-us (Japan)

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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of mouse MIP2.

The standard protein in this kit is full-length mature mouse MIP2.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), plasma (heparin), and cell culture supernatant sample types.

Urine, saliva, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY
5 ng/mL of recombinant mouse CXCL1, CXCL3, and CXCL4 were tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE
5 ng/mL of recombinant mouse CXCL1, CXCL3, and CXCL4 were tested individually for interference with 75 pg/mL of recombinant mouse MIP2(CXCL2). The % recovery of mouse MIP2 signal is shown below.

Recombinant Mouse Protein	% Recovery of MIP2 Signal
CXCL1	104%
CXCL3	103%
CXCL4	94%

SPECIES REACTIVITY
5 ng/mL of recombinant human CXCL1, CXCL2, and CXCL3 were tested for reactivity. No cross reactivity was observed.

5 ng/mL of recombinant rat CXCL2 was tested for reactivity. 3% cross reactivity was observed.

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

Other species reactivity not determined.

CALCULATION
- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence 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 chemiluminescence 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 chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence 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)	RLU		Mean RLU
	1	2	
0	255	473	364
0.46	1,465	1,454	1,460
1.37	4,729	4,065	4,397
4.12	13,502	12,138	12,820
12.4	58,941	60,291	59,616
37.0	191,480	208,330	199,905
111	544,980	552,930	548,955
333	2,266,900	1,906,600	2,086,750
1,000	6,951,800	6,123,600	6,537,700

Table 1. Example of mouse MIP2 standard curve in Sample Diluent NS. The MIP2 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	1,315	1,728	1,522
0.46	2,540	3,076	2,808
1.37	6,298	6,377	6,338
4.12	12,435	12,292	12,364
12.4	36,411	40,143	38,277
37.0	142,880	133,490	138,185
111	412,520	435,860	424,190
333	1,393,100	1,520,900	1,457,000
1,000	4,288,900	4,584,200	4,436,550

Table 2. Example of mouse MIP2 standard curve in Sample Diluent 25BP. The MIP2 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	4,643	6,851	5,747
1.37	9,598	8,767	9,183
4.12	16,014	15,344	15,679
12.4	43,818	53,017	48,418
37.0	121,060	119,640	120,350
111	379,990	411,200	395,595
333	1,037,500	1,128,800	1,083,150
1,000	3,766,500	3,654,900	3,710,700

Table 3. Example of mouse MIP2 standard curve in Sample Diluent 50BP. The MIP2 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (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
Sample Diluent NS	16	0.12 pg/mL
Sample Diluent 25BP	16	0.12 pg/mL
Sample Diluent 50BP	16	0.55 pg/mL

Recovery

Three concentrations of 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.

Native MIP2 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions for mouse serum and plasma heparin are made in Sample Diluent 50BP. Sample dilutions for mouse plasma citrate and EDTA are made in Sample Diluent 25BP. Sample dilutions for stimulated J774A.1 are made in Sample Diluent NS.

Dilution Factor	Interpolated value	100% Mouse Serum	100% Mouse Plasma (Citrate)	50% Mouse Plasma (EDTA)	100% Mouse Plasma (Heparin)	0.5% J774A.1 Stimulated cell culture supernatant
Undiluted	pg/mL	236.32	154.31	112.95	176.87	194.90
	% Expected value	**100**	**100**	**100**	**100**	**100**
2	pg/mL	121.53	76.87	52.78	98.57	102.64
	% Expected value	**103**	**100**	**93**	**111**	**105**
4	pg/mL	53.22	39.87	24.29	49.04	48.26
	% Expected value	**90**	**103**	**86**	**111**	**99**
8	pg/mL	24.81	20.54	11.95	23.95	23.10
	% Expected value	**84**	**106**	**85**	**108**	**95**
8	pg/mL	12.13	10.93	5.62	10.15	10.11
	% Expected value	**82**	**113**	**80**	**92**	**83**

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
Undiluted	pg/mL	73.50
	% Expected value	**100**
2	pg/mL	33.91
	% Expected value	**92**
4	pg/mL	19.14
	% Expected value	**104**
8	pg/mL	10.55
	% Expected value	**115**
8	pg/mL	4.16
	% Expected value	**90**

Precision

Mean coefficient of variations of interpolated values of MIP2 from three concentrations concentration of J774A.1 stimulated cell culture supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	1.64	4.87

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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