ab326401 – Mouse MAP2 SimpleStep ELISA® Kit Chemiluminescent

For the quantitative measurement of MAP2 in Mouse serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, and cell and tissue extract.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326401

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 (ab253229).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Mouse MAP2 Capture Antibody 10X	600 µL	+4°C
Mouse MAP2 Detector Antibody 10X	600 µL	+4°C
Mouse MAP2 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BR	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

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

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Method for determining protein concentration (BCA assay recommended).
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.

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.

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.

1X Cell Extraction Buffer PTR: 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.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BR. 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 5BR. 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 (recommended).

1. Reconstitute the **MAP2** protein standard by adding the volume indicated on the protein vial label. For **serum, plasma, and cell culture supernatant** samples measurements, use Sample Diluent NS. For **tissue extract** samples measurements, use 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 150,000 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 320 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-9.
4. 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**	80	320	150,000	30,000
2	Standard#1	75	150	30,000	10,000
3	Standard#2	75	150	10,000	3,333.3
4	Standard#3	75	150	3,333.3	1,111.1
5	Standard#4	75	150	1,111.1	370.4
6	Standard#5	75	150	370.4	123.5
7	Standard#6	75	150	123.5	41.2
8	Standard#7	75	150	41.2	13.7
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	≤ 50%
Plasma – Citrate*	≤ 50%
Plasma – EDTA*	≤ 50%
Plasma – Heparin*	≤ 50%
Cell Culture Media*	≤ 25%
Mouse Brain Tissue Extract	0.94 - 15.0 µg/ml

*Media is RPMI 1640 containing 10% fetal bovine serum.

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 1:2 into Sample Diluent NS and assay. Store un-diluted serum at -20ºC 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. Dilute samples 1:2 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC 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 1:4 into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets 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 4ºC. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

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 | 2026-03-02

For all technical or commercial enquiries please go to:
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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of Mouse MAP2.

The standard protein in this kit is an N-terminal fragment, a fragment representing approximately 27.8% of the full-length mass of Mouse MAP2.

Native signal was detected in tissue extract sample types.

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), plasma (heparin), and cell culture supernatant sample types.

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

SPECIES REACTIVITY

Recombinant Human MAP2 protein was tested at 15,000 pg/mL. No cross reactivity was observed.

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	5,912	5,912	5,912
13.7	9,013	7,020	8,017
41.2	15,613	16,100	15,857
123.5	40,235	39,167	39,701
370.4	66,640	66,946	66,793
1,111.1	189,220	209,150	199,185
3,333.3	576,760	693,780	635,270
10,000	2,376,200	2,364,300	2,370,250
30,000	6,626,500	6,745,100	6,685,800

Table 1. Example of Mouse MAP2 standard curve in Sample Diluent NS. The MAP2 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	3,235	3,235	3,235
13.7	6,274	6,330	6,302
41.2	10,117	9,893	10,005
123.5	22,400	23,129	22,765
370.4	52,432	54,778	53,605
1,111.1	152,830	151,760	152,295
3,333.3	493,400	480,780	487,090
10,000	1,449,400	1,464,100	1,456,750
30,000	3,865,000	4,015,300	3,940,150

Table 1. Example of Mouse MAP2 standard curve in 1X Cell Extraction Buffer PTR. The MAP2 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	8	9.5 pg/mL
1X Cell Extraction Buffer PTR	8	7.0 pg/mL

Recovery

Three concentrations of MAP2 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% Serum	84	82 - 87
25% Plasma – Citrate	84	81 - 86
50% Plasma – EDTA	84	82 - 89
50% Plasma – Heparin	91	93 - 101
25% Cell Culture Media*	93	90 - 99
7.5 ug/mL Mouse Brain Tissue extract	81	80 - 83

*Media is RPMI 1640 containing 10% fetal bovine serum.

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 MAP2 was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Mouse Serum	50% Mouse Plasma (Citrate)	50% Mouse Plasma (EDTA)	50% Mouse Plasma (Heparin)	25% Cell Culture Media
Undiluted	pg/mL	4,849.65	4,050.87	5,573.45	5,413.76	4,645.09
	% Expected value	100	100	100	100	100
2	pg/mL	2,468.44	2,112.31	2,777.45	2,827.11	2,513.57
	% Expected value	102	104	100	104	108
4	pg/mL	1,197.13	1,047.46	1,363.51	1,445.60	1,213.40
	% Expected value	99	103	98	107	105
8	pg/mL	668.16	NL	684.79	797.48	656.67
	% Expected value	110	NL	98	118	113
16	pg/mL	NL	NL	309.65	371.24	328.95
	% Expected value	NL	NL	89	110	113

NL – Non-Linear

Native MAP2 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	15 ug/mL Mouse Brain Extract
Undiluted	pg/mL	3,470.17
	% Expected value	100
2	pg/mL	1,653.38
	% Expected value	95
4	pg/mL	950.10
	% Expected value	110
8	pg/mL	466.24
	% Expected value	107
16	pg/mL	241.06
	% Expected value	111

Precision

Mean coefficient of variations of interpolated values of MAP2 from a single concentration of spiked serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	5.8	2.5

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 | 2026-03-02

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