

Mouse Mac-2 binding protein (Mac-2bp)

Assay Kit - IBL

96 Well

Please read carefully this instruction prior you use this assay kit.

INSTRUCTIONS FOR USE

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

KIT COMPONENT

1	Precoated plate: (Anti- Mouse Mac-2bp 132A1A Rat IgG)	96Well x 1
2	Labeled antibody conc.: (30X) HRP conjugated Anti- Mouse Mac-2bp 145A1A Rat IgG)	0.4mL x 1
3	Standard: (Recombinant Mouse Mac-2bp)	0.5mL x 2
4	EIA buffer	30mL x 1
5	Solution for labeled antibody	12mL x 1
6	Chromogen: TMB solution	15mL x 1
7	Stop solution	12mL x 1
8	Wash buffer conc.	50mL x 1

MEASURING SAMPLES

Mouse serum, EDTA plasma, Tissue extract and cell culture supernatant.

※Example for Preparation of Tissue Extract

- (1) Add 200μL TNE buffer (50mM Tris-HCl pH8.0, 50mM NaCl, 5mM EDTA) on 1mm a piece of tissue and homogenize it.
- (2) After centrifugation, collect the supernatant and quantitatively measure the protein mass.
- (3) Adjust the concentration to become 1mg/mL by adding the TNE buffer.

PRINCIPLE

This kit is a solid phase sandwich ELISA (Enzyme-linked Immunosorbent Assay). As a primary antibody is coated on a plate, samples and standard are added into the wells for 1st reaction. After the reaction, HRP-conjugated secondary antibody is added into the wells for 2nd reaction. After washing away unbound the secondary antibody, Tetra Methyl Benzidine (TMB) is added to the wells and color develops.

OPERATING PRECATION

- 1 Test samples should be measured soon after collection. For storage of samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
- 2 Test samples should be diluted with "4, EIA buffer" contained in this kit.
- 3 Duplicate measurement of test samples and standards is recommended.
- 4 Standard curve should run for each assay.
- 5 Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
- 6 All reagents should be brought to room temperature (R.T.) and mixed completely and gently before use. After mixing them, make sure of no change in quality of the reagents.
- 7 Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- 8 Fill the wash buffer each well, invert the plate and make sure the liquid is completely removed by shaking it off if you use a washing bottle. Repeat this washing process several times as instructed in order to avoid any insufficient washing process.
- 9 After remove the wash buffer, tapping the plate against a clean paper towel for completely removing the liquid from the wells and make sure the paper towel is not contact with inside of the wells in this process.
- 10 "6, Chromogen - TMB solution" should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
- 11 After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination.
- 12 Measurement of O.D. should be done within 30 minutes after addition of "7, Stop solution".

OPERATION MANUAL AND DOSAGES

1. Materials needed but not supplied.

Plate reader	Micropipette and tip
Test tubes for dilution	Measuring cylinder and beaker
Deionized water	Plate washer
Paper towel	Collecting container
Refrigerator	(i.e. clean disposable test tube)
Incubator(37°C ± 1°C)	

2. Preparation

- (1) Preparation of wash buffer
Dilute "8, Wash buffer conc." 40 fold with deionized water. The diluted one is used for the assay as a wash buffer. Adjust the required quantities if needed.
- (2) Preparation of labeled antibody
Dilute "2, Labeled antibody conc." 30 fold with "5, Solution for labeled antibody"

using a prepared collecting container.

Example)

In case you use one strip (8 well), the required quantity of Labeled antibody is 800 μL. (Dilute 30 μL of "2, Labeled antibody Conc." with 870 μL of "5, Solution for labeled antibody" and mix it. And use 100μL the mixed solution in each well.) This operation should be done just before applying labeled antibody.
The remaining "2, Labeled antibody Conc." should be stored at 4°C in a firmly sealed vial.

(3) Preparation of standard

Add 0.5 mL of deionized water into the vial of "3, Standard" and completely dissolve it. Concentration of the standard is 100 ng/mL. The standards enclosed in this kit can be frozen and stored after reconstitution. However the freeze-thaw shall not be repeated.
Prepare 7 test tubes for dilution of the standard and adding 230 μL of the EIA buffer into each tube.

Put 230 μL of 100 ng/mL standard into the tube 50 ng/mL (Tube-1) and gently mix it. Afterword, put 230 μL of the mixed liquid of tube-1 into the tube 0.78 ng/mL (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 7 points of diluted standard between 0.78 ng/mL and 50 ng/mL.

Tube-1	50	ng/mL
Tube-2	25	ng/mL
Tube-3	12.5	ng/mL
Tube-4	6.25	ng/mL
Tube-5	3.13	ng/mL
Tube-6	1.56	ng/mL
Tube-7	0.78	ng/mL

(4) Preparation of test samples

Dilute test samples with "4, EIA buffer" contained in this kit as follows.

Mouse serum	: 50 fold.
Mouse EDTA plasma	: 50 fold
Cell culture supernatant	: more than 4 fold
Tissue extract	: 5 fold.

3. Measurement Procedure

- (1) Add test sample blank
Determine wells for test sample blank. Put 100μL each of "4, EIA buffer" into the wells.
- (2) Add prepared test samples and standard
Put 100 μL prepared test samples and 100 μL prepared standard into appropriate wells.
- (3) Incubation with plate lid (1st reaction).
- (4) Washing
Wash the plate with the prepared wash buffer and remove all liquid.
- (5) Add prepared labeled antibody
Put 100 μL prepared labeled antibody into the wells.
- (6) Incubation with plate lid (2nd reaction).
- (7) Washing
Wash the plate with the prepared wash buffer and remove all liquid completely.
- (8) Add "6, Chromogen - TMB solution"
Put 100 μL the TMB solution into the wells.
- (9) Incubation in dark
- (10) Add "7, Stop solution"
Put 100 μL the Stop solution into the wells.
- (11) Determination of optical density (O.D.)
Remove any dirt or drop of water on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, measure the both O.D. of standard and the test samples against a test sample blank.
Measurement wavelength: 450 nm. In case of 2 wavelengths:
Main wavelength is 450nm. Sub-wavelength is between 600 and 650 nm.

Table for measurement procedure

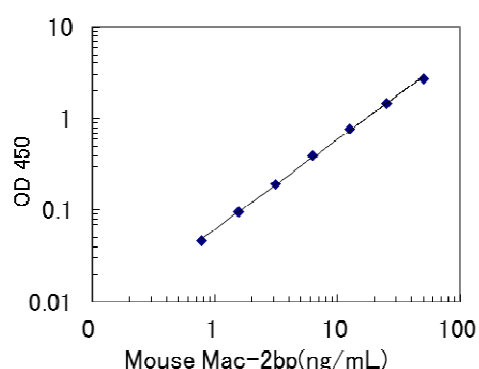
	Test samples	Standard	Test sample blank
Reagents	Test samples 100 μL	Diluted Standard 100 μL	EIA buffer 100 μL
1st reaction	Incubation for 60 minutes at 37°C with plate lid		
Washing	4 times (wash buffer more than 350 μL)		
Labeled antibody	100 μL	100 μL	100 μL
2nd reaction	Incubation for 30 minutes at 2~8°C with plate lid.		
Washing	5 times (wash buffer more than 350 μL)		
TMB solution	100 μL	100 μL	100 μL
Chromogenic reaction	Incubation for 30 minutes at R.T. (shielded).		
Stop solution	100 μL	100 μL	100 μL
Measuring O.D.	450 nm / 600~650 nm		

CALCULATION OF TEST RESULT

- 1 Plot the concentration of the standard on the x-axis and its O.D. on the y-axis. Draw a standard curve by applying appropriate regression curve on each plot (i.e. quadratic regression of double logarithm conversion).
- 2 Read the concentration by applying the absorbance of the test samples on a standard curve.
- 3 Calculate the concentration of the test samples by multiplying dilution ratio of test samples on the value.

Example of standard curve and measured value

標準品濃度 (ng/mL)	吸光度 (450nm)
50.	2.716
25.	1.472
12.5	0.774
6.25	0.400
3.13	0.195
1.56	0.097
0.78	0.046



PERFORMANCE AND CHARACTERISTICS

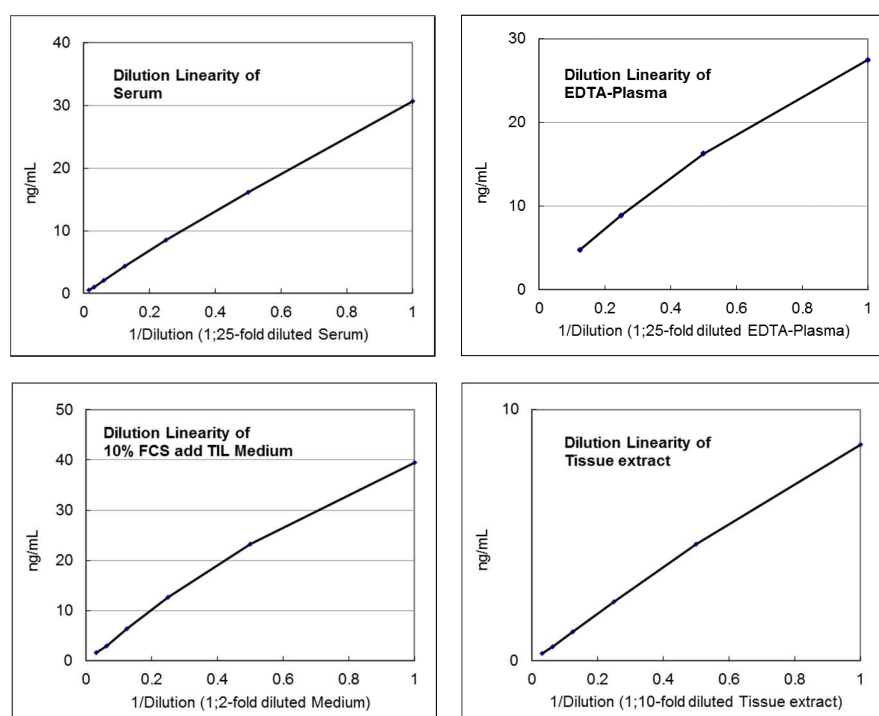
1 Sensitivity

0.09 ng/mL

2 Measurement range

0.78 ~ 50 ng/mL

3 Dilution linearity



4 Added recovery assay

Specimen	Additive Amount (ng/mL)	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
Mouse Serum (x50)	12.50	27.13	23.74	87.5
	3.13	17.76	17.30	97.4
	0.78	15.41	15.56	101.0
Mouse EDTA-Plasma (x50)	12.50	18.76	17.72	94.5
	3.13	9.38	9.08	96.8
	0.78	7.04	6.87	97.6
10% FCS added TIL Media (x4)	25.00	25.00	22.80	91.2
	6.25	6.25	6.19	99.0
	1.56	1.56	1.39	89.1
Tissue extract (x5)	12.50	28.11	24.30	86.4
	3.13	18.74	18.34	97.9
	0.78	16.40	16.12	98.3

5 Intra-assay

Measurement value (ng/mL)	SD (ng/mL)	CV (%)	n
27.19	0.55	2.0	24
8.30	0.30	3.6	24
2.48	0.09	3.6	24

6 Inter-assay

Measurement value (ng/mL)	SD (ng/mL)	CV (%)	n
26.54	0.85	3.2	6
8.10	0.14	1.7	6
2.72	0.19	7.0	6

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1 Precaution for handling (Hazard prevention)

- (1) Treat the components carefully and wash hands after handling it.
- (2) "7, Stop solution" is a strong acid substance (1N Sulfuric acid). Therefore, it should be careful for the treatment and do not contact your skin and clothes with it. It also needs to pay attention to the disposal of it.

2 Precaution for intended use

- (1) "3, Standard" is lyophilized products. It should be careful to open this vial.
- (2) All reagents should be stored at 2~8°C.
- (3) Precipitation can be seen in "4, EIA buffer", "5, Solution for labeled antibody" and "8, Wash buffer conc.", however, it does not affect its performance.
- (4) Do not mix or replace the reagents with the reagents from a different lot or kit.
- (5) Do not use expired reagents.

3 Precaution for disposal

- (1) Dispose used materials after rinsing them with large quantity of water.

The expiry date is specified on the outer box.

PACKAGE UNIT AND PRODUCT NUMBER

Package unit: 96 Well
Product number: 27796

REFERENCE

1. Iwata A, Kamada Y, Ebisutani Y, Yamamoto A, Ueda Y, Arai H, Fujii H, Takamatsu S, Maruyama N, Maeda M, Takehara T, Miyoshi E. Establishment of mouse Mac-2 binding protein enzyme-linked immunosorbent assay and its application for mouse chronic liver disease models. *Hepato Res.* 2016 Sep 11.*

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