Rat Osteopontin 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-Rat OPN(O-17) Rabbit IgG A.P.)	96Well x 1
2	**Labeled antibody conc.:** (30X) HRP conjugated Anti-Rat OPN(O-165) Rabbit IgG Fab' A.P.)	0.4mL x 1
3	**Standard:** (Recombinant Rat OPN)	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

Rat EDTA-plasma, urine and cell culture supernatant

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 Osteopontin (OPN) is an extremely unstable protein. It should be stored under -80 °C if it is not measured soon after the collection. It should be thawed at low temperature and mix it well prior measuring. It should be also avoided to repeat freeze-thaw as the measurement value would be affected and the value become low.
2 Serum and heparin-plasma are not recommended to use because the thrombin cleavage site is existed in rat OPN. Accurate values would not be obtained if serum or heparin-plasma is used for the assay.
3 It is recommended to dilute by PBS for rat EDTA-plasma or urine in advance. The indicated dilution ratio is 10 fold, however, additional dilution and repeating the assay might be required if the value is not fit in the measuring range.
4 Since full length OPN in urine would be nearly non-existent in some rat strains, the value would be below the limited sensitivity even if the sample applies directly. Dilution ratio for measuring rat urine sample should be carefully determined by an individual user prior the assay is conducted.
5 Since OPN in urine is easily broken down, it is recommended to add a protease inhibitor such as PMSF in the sample.
6 Dilution ratio for measuring rat OPN in cell culture supernatant should be determined by an individual user because OPN expressed volume might be different depend on samples.
7 Rat OPN cleaved by Thrombin cannot be measured by this assay kit.
8 Both recombinant and native rat OPN can be measured.
9 Duplicate measurement of test samples and standards is recommended.
10 Standard curve should run for each assay.
11 Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
12 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.
13 Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
14 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.
15 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.
16 "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.
17 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.
18 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
Test tubes for dilution
Micropipette and tip
Measuring cylinder and beaker
Deionized water
Paper towel
Incubator (37°C±1°C)
Plate washer
Collecting container
(i.e. clean disposable test tube)
Refrigerator

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.

(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 9.5 ng/mL.
Prepare 7 test tubes for dilution of the standard and adding 230 µL of the EIA buffer into each tube.

Put 230 µL of 9.5 ng/mL standard into the tube 4.75 ng/mL (Tube-1) and gently mix it. Afterword, put 230 µL of the mixed liquid of tube-1 into the tube 2.38 ng/mL (Tube-2) and gently mix it. Dilute two fold standard solution in series to set up 7 points of diluted standard between 4.5 ng/mL and 0.15 ng/mL.

Tube-1	4.75 ng/mL
Tube-2	2.38 ng/mL
Tube-3	1.19 ng/mL
Tube-4	0.59 ng/mL
Tube-5	0.30 ng/mL
Tube-6	0.15 ng/mL
Tube-7	0.07 ng/mL

(4)Preparation of test samples

Rat EDTA-plasma and urine samples should be diluted more than 10 fold.
Rat cell culture supernatant should be diluted more than 2 hold.

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		

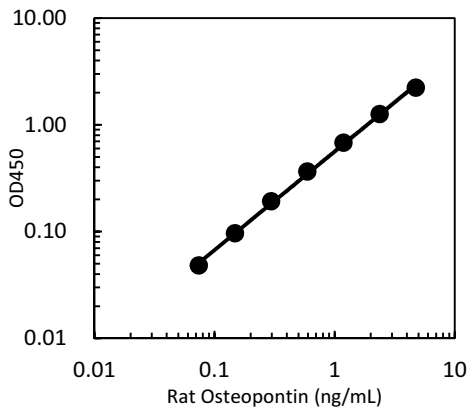
Manufacturer: Immuno-Biological Laboratories Co., Ltd. URL: http://www.ibl-japan.co.jp E-mail: do-ibl@ibl-japan.co.jp

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

Standard (ng/mL)	O.D. (450nm)
4.75	2.222
2.38	1.258
1.19	0.677
0.59	0.364
0.30	0.192
0.15	0.096
0.07	0.048



PERFORMANCE AND CHARACTERISTICS

1 Sensitivity
0.01 ng/mL

2 Measurement range
0.07 ～ 4.75 ng/mL

3 Dilution linearity

Test samples	Dilution Ratio (x)	Theoretical value (ng/mL)	Measurement value (ng/mL)	%
10% FCS Added RPMI-1640	2	1.19	1.11	93.3
	4	0.60	0.59	98.3
	8	0.30	0.30	100.0
Rat Plasma (EDTA)	6	3.39	2.72	80.2
	12	1.71	1.78	104.1
	24	0.89	0.93	104.5
Rat Urine	1000	1.59	1.75	110.1
	2000	0.79	0.86	108.9
	4000	0.39	0.41	105.1

4 Added recovery assay

Test samples	Theoretical value (ng/mL)	Measurement value (ng/mL)	%
10% FCS Added RPMI-1640 (x2)	1.19	0.98	82.4
	0.60	0.53	88.3
	0.30	0.26	86.7
Rat Plasma (EDTA) (x10)	2.76	2.26	81.9
	2.17	2.29	105.5
	1.87	2.15	115.0
Rat Urine (x1000)	1.60	1.62	101.3
	1.01	1.12	110.9
	0.71	0.78	109.9

5 Intra-assay

Measurement value (ng/mL)	SD (ng/mL)	CV (%)	n
3.11	0.10	3.2	23
0.72	0.04	5.6	23
0.22	0.01	4.5	23

6 Inter-assay

Measurement value (ng/mL)	SD (ng/mL)	CV (%)	n
3.18	0.16	5.0	35
0.73	0.04	5.5	35
0.22	0.01	4.5	35

7 Specificity

Substance	Cross reactivity (%)
Rat OPN	100
Human OPN	≦ 0.1
Mouse OPN	≦ 0.1

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.

STORAGE AND THE TERM OF VALIDITY

Storage Condition: 2 - 8°C
The expiry date is specified on the outer box.

PACKAGE UNIT AND PRODUCT NUMBER

Package unit: 96 Well
Product number: 27360

CONTACT DETAILS

Immuno-Biological Laboratories Co., Ltd.
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