DRG® Osteopontin (rat) (EIA-4934)

As of 30 Dec. 2008 (Vers. 1.0) **For Veterinary Use Only**

INTRODUCTION

Osteopontin (OPN) is a secreted glycoprotein that was originally isolated from bone. At present, it is known as a highly acidic calcium-binding glycosylated phosphoprotein secreted by many cell types, including osteoblasts, kidney tubule cells, macrophages, activated T cells, and vascular smooth muscle cells. Its molecular weights have been reported in the range of 66 kDa to 44 kDa depending on glycosylation and phosphorylation.

One important feature of OPN is that it contains an Arg-Gly-Asp (RGD) amino acid sequence. This motif is present in fibronectin, vitronectin and a variety of other extra cellular proteins that bind members of the integrin family of cell surface receptors such as αvβ3.

Another important of OPN is the presence of various molecular forms in vivo due to differential RNA splicing, glycosylation, phosphorylation, sulfation, and susceptibility to proteases. Both OPN and thrombin are likely to be localized together at the site of injury, in- flammation, and angiogenesis and in tumor tissues. Osteopontin is susceptible to proteolytic fragmentation, and this process may have physiologic importance. A report demonstrated that thrombin treatment enhanced OPN cell adhesive activity, suggesting that cleavage of OPN by thrombin exposes a cryptic adhesive sequence. And then, it was shown that an aminoterminal OPN fragment contains a cryptic binding site that can be recognized by α9β1 integrin. Furthermore, OPN contains multiple cell binding sites and interacts with various receptors; these interactions may have distinct functional.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Rat OPN.

MEASUREMENT RANGE

0.07 - 4.75 ng/mL

INTENDED USE

- This kit is to be used for the in-vitro quantitative determination of Rat Osteopontin (Rat OPN) in plasma (EDTA), urine, or cell culture media. Please store all samples at –80 °C before use because OPN molecule is unstable protein.
- The recommend dilution for rat EDTA plasma samples is more than 10 fold by PBS. Please assay again with more dilution if the assay with dilution of more than 10 fold take range over the high standard value.
- The assay by serum or heparin plasma samples are discouraged, because OPN is easily cleaved by thrombin and has several heparin binding sites in the molecule. Therefore, serum won't give correct value and heparin plasma will give any effect in the assay.
- The recommended dilution for urine sample varies by strain of rat, therefore, the dilution rate should be optimized by each laboratory. In some strains, there are almost no full-length molecule of OPN in urine and could not detect with this ELISA. Since OPN in urine sample is easy to be degraded, we recommend adding some protease inhibitor such as PMSF. Moreover, when it cannot measure immediately after collection, please store at -80 °C or less. Since measured value falls by repetition of freeze/thaw cycles, cautions are required.

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- The recommend dilution for cell culture media samples is various by expression level of OPN, therefore, the dilution rate should be optimized by each laboratories.
- The kit can not assay thrombin-cleaved Rat OPN.
- Both recombinant and native forms of Rat OPN can be detected with the kit.

KIT COMPONENT

o **Precoated plate** :	Anti-Rat OPN (O-17) Rabbit IgG Affinity Purify	96 Wells x 1
o Labeled **Antibody** Conc. : (30X)HRP conjugated Anti- Rat OPN (O-165) Rabbit IgG Fab' Affinity Purify		0.4 mL x 1
o **Standard** :	Recombinant Rat OPN	0.5 mL x 2
o **EIA Buffer** :	1% BSA, 0.05% Tween20 in PBS	30 mL x 1
o **Antibody Diluent**: (Solution for Labeled antibody) 1% BSA, 0.05% Tween20 in PBS		12 mL x 1
o **Chromogen** :	TMB solution	15 mL x 1
o **Stop Solution** :	1N H_2SO_4	12 mL x 1
o **Wash Buffer** Conc. :	(40X) 0.05% Tween20 in phosphate buffer	50 mL x 1

OPERATION MANUAL

1.1 Materials needed but not supplied

- Plate reader (450 nm)
- Graduated cylinder and beaker ·
- Refrigerator (as 4°C) ·
- Paper towel ·
- Incubator (37°C ± 1°C) ·
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Deionized water
- Graph paper (log/log)
- Tube for dilution of Standard PBS (for sample dilution)

1.2 Preparation

1) Preparation of wash buffer

"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "Washing buffer Conc." to room temperature and then, mix it gently and completely before use.

Dilute 50 mL of "Wash buffer Conc." with 1,950 mL of deionized water and mix it.

This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.

2) Preparation of Labeled antibody

"2, Labeled antibody Conc." is a concentrated (30X). Dilute "Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube.

Use this resulting solution as Labeled antibody.

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 the resulting solution by 100 µL in each well.)

This operation should be done just before the application of Labeled antibody.

The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.

3) Preparation of Standard

Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely.

This solution is 9.5 ng/mL Rat OPN standard.

4) Dilution of Standard

Prepare 8 tubes for dilution of "3, Standard".

Put 230 µL each of "4, EIA buffer" into the tube. Specify the following concentration of each tube."

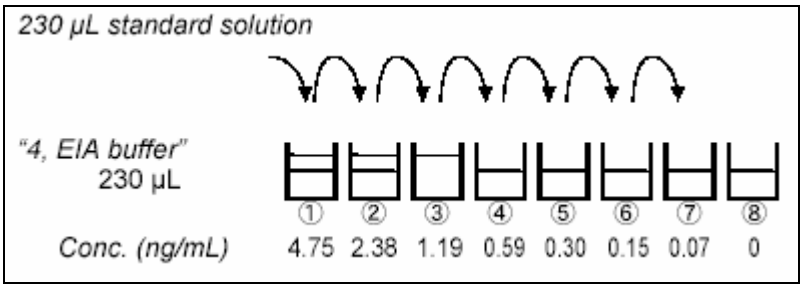
Tube-1	4.75 ng/mL	
Tube-2	2.38 ng/mL	
Tube-3	1.19 ng/mL	
Tube-4	0.59 ng/mL	
Tube-5	0.30 ng/mL	
Tube-6	0.15 ng/mL	
Tube-7	0.07 ng/mL	
Tube-8	0 ng/mL	(Test Sample Blank)

Put 230 µL of Standard solution into tube-1 and mix it gently.

Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 4.75 ng/mL and 0.07 ng/mL.

Tube-8 is the test sample blank as 0 ng/mL.

See following picture.



5) Dilution of test sample

Test sample may be diluted with "4, EIA buffer" or PBS as necessary.

If the concentration of Rat OPN in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

1.3 Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents.

Standard curve shall be prepared simultaneously with the measurement of test samples.

1. Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
2. Determine wells for test sample blank, test sample and diluted standard.
   Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
3. Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
4. Wash each well of the precoated plate vigorously with wash buffer using the washing bottle. Then, fill each well with wash buffer and leave the precoated plate laid for 15-30 seconds. Remove wash buffer completely from the precoated plate by snapping.
   This procedure must be repeated more than 7 times.
   Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
   In case of using a plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
5. Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6. Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
7. Wash the precoated plate 9 times in the same manner as 4).
8. Take the required quantity of "6, Chromogen" into a disposable test tube.
   Then, pipette 100 µL from the test tube into the wells.
   Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
9. Incubate the precoated plate for 30 minutes at room temperature in the dark.
   The liquid will turn blue by addition of "6, Chromogen".

10. Pipette 100 µL of "7, Stop Solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by addition of "7, Stop solution".
11. Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank. The measurement shall be done within 30 minutes after addition of "7, Stop solution".

	Test Sample	Standard	Test Sample Blank	Reagent Blank
Reagents	Test sample 100 µL	Diluted standard (Tube 1~7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at 37°C with plate lid				
Washing 7 times				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C with plate lid				
Washing 9 times				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

SPECIAL ATTENTION

1. Test samples should be measured soon after collection. For the storage of test 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" or PBS, if the need arises.
3. Duplicate measurement of test samples and standard is recommended.
4. Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5. Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
6. Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7. "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.

8. Measurement should be done within 30 minutes after addition of "7, Stop solution".
9. Adding some protease inhibitor such as PMSF to urine samples is recommended to avoid cleavage of OPN. Moreover, when it cannot measure immediately after collection, please store at -80 °C or less. Since measured value falls by repetition of freeze/thaw cycles, cautions are required.
10. Please perform plasma by EDTA blood collecting. Moreover, when it cannot measure immediately after collection, please store at -80°C or less. Since measured value falls by repetition of freeze/thaw cycles, cautions are required.

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting.

Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper.

Draw the best smooth curve through these points to construct the standard curve.

Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc.	Absorbance
(ng/mL)	(450nm)
4.75	2.237
2.38	1.273
1.19	0.692
0.59	0.379
0.30	0.207
0.15	0.111
0.07	0.063
0 (Test Sample Blank)	0.015

The typical standard curve is shown above. This curve cannot be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1.4 Titer Assay

(Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value (ng/mL)	Theoretical Value (ng/mL)	%
10%FCS added RPMI-1640	2	1.11	1.19	93.3
	4	0.59	0.60	98.3
	8	0.30	0.30	100.0
Rat Plasma (EDTA)	6	2.72	3.39	80.2
	12	1.78	1.71	104.1
	24	0.93	0.89	104.5
Rat Urine	1,000	1.75	1.59	110.1
	2,000	0.86	0.79	108.9
	4,000	0.41	0.39	105.1

1.5 Added Recovery Assay

Specimen	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 (x1,000)	1.60	1.62	101.3
	1.01	1.12	110.9
	0.71	0.78	109.9

1.6 Intra - Assay

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

1.7 Inter - Assay

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

1.8 Specificity

Compound	Cross Reactivity
Rat Osteopontin	100 %
Human Osteopontin	≤ 0.1 %
Mouse Osteopontin	≤ 0.1 %

1.9 Sensitivity

0.01 ng/mL

The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. “1, Precoated plate” and “3, Standard” contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid production of explosive metallic azide.

5. Precipitation may occur in "2, Labeled antibody Conc.", however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C

The term of validity : 12 months (The expiry date is specified on outer box.)

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..