Code No. 27363

Rat DMP1 Assay Kit - IBL

INTRODUCTION

Dentin Matrix Protein 1(DMP1) is a constitutive protein in extracellular matrix of osteocyte and it belongs to Small Integrin-Binding Ligand, N-linked Glycoprotein (SIBLING) family. It is synthesized as a full-length 105kDa proprotein and it is divided into 37kDa at N terminal side and 57kDa at C terminal side in the secretion process. It has a lot of acidic domains and it binds with calcium because of the feature that become negative charged within tissues and it has an important role for bone mineralization.

DMP1 expresses in osteocyte while other constitutive proteins in extracellular matrix such as Osteopontin, Osteocalcin and Bone sialoprotein express in osteoblast cells.

Osteocyte which is a main constituent cell of bone regulates activities of osteoclastic cells and osteoblast cells and it adjusts bone remodeling process. It also acts as an endocrine cell which secrets FGF-23. DMP1 in blood is considered as a candidate of biomarker which reflects activities of osteocyte and it is also suggested to become useful information for research of bone metabolism and phosphate metabolism.

The concentration of DMP1 in rat blood can be quantitatively measured by this ELISA kit.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Rat DMP1.

MEASUREMENT RANGE

39.06 – 2500 pg/mL

INTENDED USE

For research use only, not for use in diagnostic procedures.

This IBL's assay kit is capable for the quantitative determination Rat DMP1 in serum, EDTA plasma and cell culture supernatant. Recommended dilution ratio for normal rat serum and EDTA plasma is around 40-fold.

KIT COMPONENT

1	Precoated plate : Anti-Rat DMP1 (90) Rabbit IgG A.P.	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti-Rat DMP1 (149) Rabbit IgG Fab' A.P.	0.4mL x 1
3	Standard : Recombinant Rat DMP1	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

OPERATION MANUAL

1. Materials needed but not supplied

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

2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1950 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 "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30-fold 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.)
<u>This operation should be done just before applying labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 2 - 8°C in firmly sealed vial.

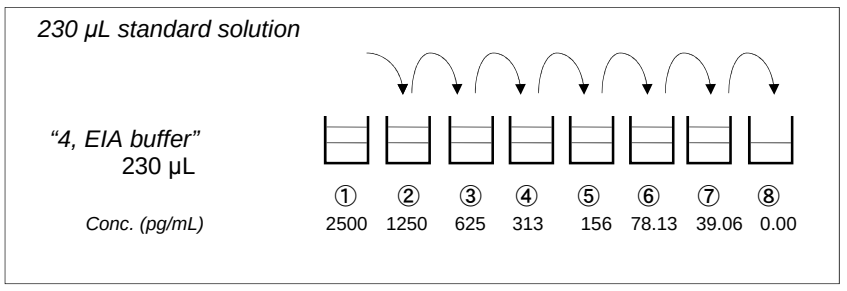
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 5000 pg/mL Rat DMP1 standard.

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	2500 pg/mL	
Tube-2	1250 pg/mL	
Tube-3	625 pg/mL	
Tube-4	313 pg/mL	
Tube-5	156 pg/mL	
Tube-6	78.13 pg/mL	
Tube-7	39.06 pg/mL	
Tube-8	0.00 pg/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 2500 pg/mL and 39.06 pg/mL. Tube-8 is the test sample blank as 0.00 pg/mL.

See following picture.



5) Dilution of test sample
Test samples should be diluted with "4, EIA buffer" suitably.
Recommended dilution ratio for normal rat serum and EDTA plasma is around 40-fold.

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.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation 60min at 37 °C with plate lid				
4 times (wash buffer more than 350 µL)				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 2 - 8°C with plate lid				
5 times (wash buffer more than 350 µL)				
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.				

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 60 minutes at 37°C after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid.*
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 2 - 8°C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid.*
8) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 µL from the test tube into every well. Please do not return the rest of used Chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution of Chromogen will turn blue.
10) Add 100 µL of "7, Stop solution" to all wells. Mix the solution by tapping the side of precoated plate. The solution 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 solution. 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".

SPECIAL ATTENTION

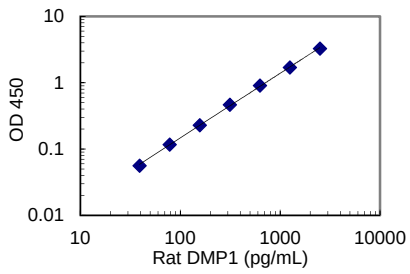
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer", suitably.
3) 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 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. Avoid contact of Chromogen with metals.
8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

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

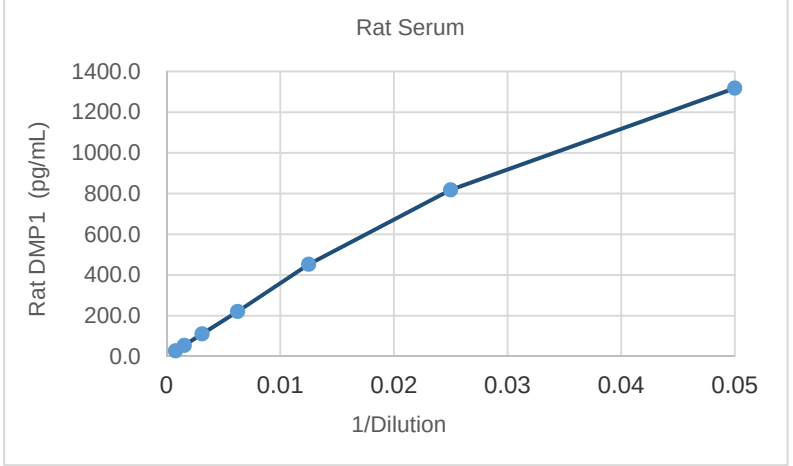
Conc. (pg/mL)	Absorbance (450nm)
2500	3.270
1250	1.708
625	0.908
313	0.468
156	0.231
78.13	0.119
39.06	0.059
0.00 (Test Sample Blank)	0.003

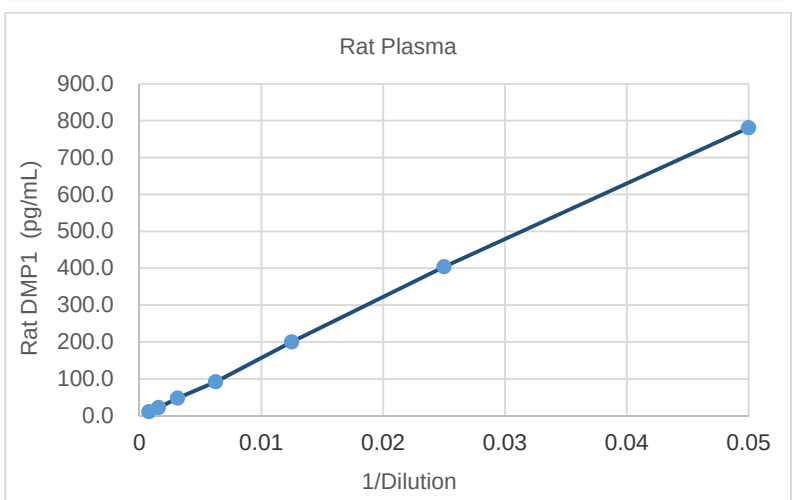


* The typical standard curve is shown above. This curve cannot be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay





2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measured Value (pg/mL)	%
Rat Serum x40	959	889	92.7
	881	839	95.2
	842	764	90.7
Rat Plasma (EDTA) x40	538	487	90.5
	460	424	92.2
	421	386	91.8
Medium (with10%FBS) X2	315	314	99.9
	159	155	97.5
	80.34	77.64	96.6

3. Intra - Assay

Mean Value (pg/mL)	SD (pg/mL)	CV (%)	n
1049	59.39	5.7	24
307	13.64	4.5	24
81.54	3.74	4.6	24

4. Inter - Assay

Mean Value (pg/mL)	SD (pg/mL)	CV (%)	n
1110	36.36	3.3	8
319	13.29	4.2	8
87.70	5.31	6.1	8

5. Specificity

Substance	Cross-Reactivity
Rat DMP1	100%
Human DMP1	N.D.
Mouse DMP1	N.D.

6. Sensitivity

4.04 pg/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. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in “2, Labeled antibody Conc.”, “4, EIA buffer” or “8, Wash buffer 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 expiry date is specified on outer box.

REFERENCE

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2. Toyosawa S, Shintani S, Fujiwara T, Ooshima T, Sato A, Ijuhin N, Komori T. Dentin matrix protein 1 is predominantly expressed in chicken and rat osteocytes but not in osteoblasts. J Bone Miner Res. 2001 Nov;16(11):2017-26.

3. Qin C, Brunn JC, Cook RG, Orkiszewski RS, Malone JP, Veis A, Butler WT. Evidence for the proteolytic processing of dentin matrix protein 1. Identification and characterization of processed fragments and cleavage sites. J Biol Chem. 2003 Sep 5;278(36):34700-8. Epub 2003 Jun 17.

4. Fisher LW, Torchia DA, Fohr B, Young MF, Fedarko NS.Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin.Biochem Biophys Res Commun. 2001 Jan 19;280(2):460-5.

5. Dallas SL, Bonewald LF.Dynamics of the transition from osteoblast to osteocyte.
Ann N Y Acad Sci. 2010 Mar;1192:437-43. doi: 10.1111/j.1749-632.2009.05246.x. Review.

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Made in Japan.