NEW
STORAGE
INSTRUCTIONS

DetectX®

Human Osteopontin
Enzyme Immunoassay Kit

Catalog Number K021-H1

Sample Types Validated:

EDTA Plasma, Urine, Milk and Tissue Culture Media

Please read this insert completely prior to using the product.

New! DualRead™ Assay
Extended Standard Curve Range

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Background

Osteopontin (OPN) is an acidic glycine-arginine-glycine-aspartate-serine containing phosphoprotein. This sequence is an integrin-binding motif common to many extracellular matrix (ECM) proteins, which can mediate cell attachment[1]. OPN has been called bone sialoprotein I, secreted phosphoprotein 1, uropontin, 2ar, and early T-lymphocyte activation factor. The human OPN gene occurs on the long arm of the chromosome 4 (4q21–4q25).

OPN has an important role in physiological and pathological mineralization, accelerated blood vessel formation, enhanced cell survival, acute and chronic inflammation[2], and it inhibits the expression of inducible nitric oxide synthase (iNOS) in both macrophages and primary renal tubular epithelial cells to exert important protective effects on tissues[3]. It is also a key molecule in neoplastic transformation and cancer development in a variety of tumors.

Phosphorylation, glycosylation and calcium modifications allow intact and fragmented OPN to direct a variety of responses including tissue remodeling, inflammation and cell survival[4]. Plasma OPN has been shown to be a positive indicator of colon and lung cancers as well as metastatic carcinomas[4-9]. The notable presence of OPN in a variety of tumors is strongly correlated to pathological stage, suggesting its critical role in tumor invasiveness, progression and metastasis[10,11]. In addition, OPN inhibits inducible nitric oxide synthase activity, thereby protecting tumor cells from NO-mediated macrophage cytotoxic attack[12].

Recently it has been shown that OPN mRNA expression increases 37-40 fold in infarct tissue after a myocardial infarction[13]. OPN-mediated myofibroblast differentiation, collagen I expression and decreased MMP expression and activity may help improve the strength of the infarct scar. Other functions of OPN, such as modulation of cardiac fibroblast growth, adhesion and spreading, may also have significant role in myocardial remodeling post-MI by maintaining the cell mass at the site of injury[14]. OPN is found in atherosclerotic plaques and may drive a number of diabetic vascular pathologies[15].

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3. Mazzali M, et al., QJM, 2002;95:3–13.
4. D.T. Denhardt, et al., J. Clin. Invest., (2001) 107: 1055-1061.
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12. A.B. Tuck, et al., Arch. Pathol. Med., (1997) 121: 578-584.
13. Trueblood NA, et al. Circ Res 2001;88:1080–7.
14. M. Singh, et al., .Mol. and Cell. Card. 48 (2010) 538–543.
15. M. Takemoto, et al., Atherioscler, Thomb. Vasc. Biol., (2000) 20: 624-628.

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Assay Principle

The DetectX® Human Osteopontin kit is designed to quantitatively measure human Osteopontin present in biological samples and tissue culture media. Please read the complete kit insert before performing this assay. A human Osteopontin standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with a monoclonal antibody to capture the Osteopontin present. After a 60 minute incubation, the plate is washed and a peroxidase conjugated Osteopontin monoclonal antibody is added. The plate is again incubated for 60 minutes and washed. Substrate is then added to the plate, which reacts with the bound Osteopontin Antibody Conjugate. After a third incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of the Osteopontin in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

Related Products

KITS

Urinary Creatinine Detection Kits	K002-H1/H5
Cyclic AMP Direct Colorimetric EIA Kits	K019-H1/H5
Cyclic AMP Direct Chemiluminescent CLIA Kits	K019-C1/C5
PKA (Protein Kinase A) Colorimetric Activity Kit	K027-H1
Histone Demethylase Activity kit	K010-F1

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Supplied Components

Clear Coated 96 Well Plate One Plate Catalog Number C075-1EA
Clear plastic microplate with break-apart strips coated with mouse anti-human Osteopontin.

Osteopontin Standard 2 Vials Catalog Number C077-1EA
Two vials of recombinant human Osteopontin at 20 ng.
Must be stored at -20°C

DetectX® Osteopontin Conjugate 5 mL Catalog Number C125-5ML
A monoclonal antibody to human Osteopontin labeled with peroxidase.

Assay Buffer Concentrate 28 mL Catalog Number X112-28ML
A 5X concentrate that should be diluted with deionized or distilled water.

Wash Buffer Concentrate 30 mL Catalog Number X007-30ML
A 20X concentrate that should be diluted with deionized or distilled water.

TMB Substrate 11 mL Catalog Number X019-11ML

Stop Solution 5 mL Catalog Number X020-5ML
A 1N hydrochloric acid solution. **Caustic.**

Plate Sealer 1 each Catalog Number X002-1EA

Storage Instructions

All components of this kit should be stored at -20°C until the expiration date of the kit.

Once opened, the kit can be stored at 4°C up to the expiration date on the kit box label, except for the Osteopontin Standard which must be stored at -20°C.

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Other Materials Required

Distilled or deionized water.

Polypropylene or glass test tubes.
Note: The use of glass test tubes reduces the observed signal by approximately 20%.

Repeater pipet and disposable tips capable of dispensing 50 and 100 µL.

A microplate washer.

Colorimetric 96 well microplate reader capable of reading optical density at 450 and 650 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

DualRead™ System

This kit uses our unique DualRead™ system. We include instructions for an alternative high standard which would typically generate ODs at 450 nm too high to be read on most plate readers. By reading the plate at 650 nm (where TMB optical density is about 3 fold lower) immediately before addition of the Stop Solution some samples outside the normal standard curve range can be read. See instructions on pages 8-10.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

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Sample Types

This assay has been validated for human EDTA plasma, urine, milk and tissue culture media (TCM) samples only. Samples containing visible particulate should be centrifuged prior to using.

The use of serum or heparin plasma is not recommended as OPN is likely to be proteolytically cleaved in these samples.

This assay detects human OPN. Mouse OPN does not cross react with this assay and murine samples cannot be analyzed using this kit.

Sample Preparation

EDTA plasma samples must be diluted ≥ 1:20 with the provided Assay Buffer prior to running in the kit. This recommended dilution will allow detection of most normal plasma samples. It may be necessary to dilute high or diseases state plasmas greater than ≥ 1:100.

Urine samples must be diluted ≥ 1:10 with the provided Assay Buffer prior to running in the kit. This recommended dilution will allow detection of most normal urine samples. It may be necessary to dilute high or diseases state urines greater than ≥ 1:40.

Milk samples should be clarified prior to being run. Centrifuge the sample at 10,000 rcf for 15 minutes at 4°C. Using a plastic pipet tip pierce the top layer on the centrifuged sample and remove the lower supernatant. Repeat the centrifugation and supernatant isolation once more. Milk samples must be diluted with the provided Assay Buffer prior to running in the kit. A dilution of 1:2,000 or greater is recommended to detect most milk samples within the standard curve range.

TCM samples should be diluted in TCM and read off a standard curve generated in the same TCM or diluted ≥1: 20 in Assay Buffer and read off a standard curve generated in Assay Buffer.

Any samples with Osteopontin concentrations outside the standard curve range should be diluted further with Assay Buffer or TCM, as appropriate, to obtain readings within the standard curve range.

It is up to the end user to determine the appropriate dilution for their samples.

Use all samples within 2 hours of dilution.

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Reagent Preparation

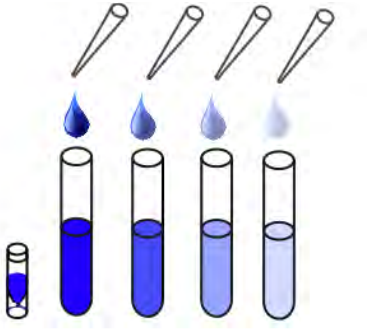
Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Osteopontin concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Wash Buffer
Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

Standard Preparation
Add 500 µL of Assay Buffer to one vial of OPN standard to generate a 40 ng/mL Stock. Label six test tubes as #1 through #6. Pipet 250 µL of Assay Buffer into tubes #1 to #6. Add 250 µL of the 40 ng/mL Osteopontin stock solution to tube #1 and vortex completely. Take 250 µL of the Osteopontin solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of Osteopontin in tubes #1 through #6 will be 20, 10, 5, 2.5, 1.25, and 0.625 ng/mL.

Use all Standards within 2 hours of preparation.



	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer Volume (µL)		250	250	250	250	250	250
Addition	**Vial**	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Volume of Addition (µL)	**500**	250	250	250	250	250	250
Final Conc (ng/mL)	**40**	20	10	5	2.5	1.25	0.625

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Assay Protocol

NOTE: If you believe that any of your samples may have high OPN levels in them (such as milk samples) we would recommend using the 40 ng/mL Stock OPN as an additional standard. In this case the assay must be read using the DualRead™ system by reading the plate at 650 nm prior to addition of stop solution.

1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

2. Pipet 50 µL of samples or standards into wells in the plate. Pipet 50 µL of Assay Buffer into the zero standard wells.

3. Incubate at room temperature for 60 minutes. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.

4. Add 50 µL of the DetectX® Osteopontin Conjugate to each well, using a repeater pipet.

5. Incubate at room temperature for 60 minutes.

6. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.

7. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.

8. Incubate the plate at room temperature for 30 minutes.

   DualRead™
   If the blue substrate color of any of your samples appears darker than the 20 ng/mL standard, we recommend reading the plate at 650 nm one minute prior to adding stop solution.

9. Add 50 µL of the Stop Solution to each well, using a repeater pipet.

10. Read the optical density generated from each well at 450 nm.

11. Use the plate reader's built-in 4PLC software capabilities to calculate Osteopontin concentration for each sample.

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Calculation of Results

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations should be multiplied by the dilution factor to obtain neat sample values.

Typical Data

Sample	Mean OD (650nm)	Mean OD (450 nm)	Human Osteopontin Conc. (ng/mL)
Alt. Standard	1.813	-	40
Standard 1	0.797	1.987	20
Standard 2	0.262	0.662	10
Standard 3	0.111	0.284	5
Standard 4	0.067	0.144	2.5
Standard 5	0.051	0.094	1.25
Standard 6	0.046	0.076	0.625
B0	0.040	0.057	0
Sample 1	0.627	1.859	17.1 (650 nm)/19.2 (450 nm)
Sample 2	0.284	0.631	10.4 (650 nm)/9.53 (450 nm)

Always run your own standard curve for calculation of results.
Do not use this data.

Optional optical density measurement at 650 nm can be performed if any samples appear to generate blue color with TMB that would be in excess of the 20 ng/mL OPN standard. See curve on page 11.

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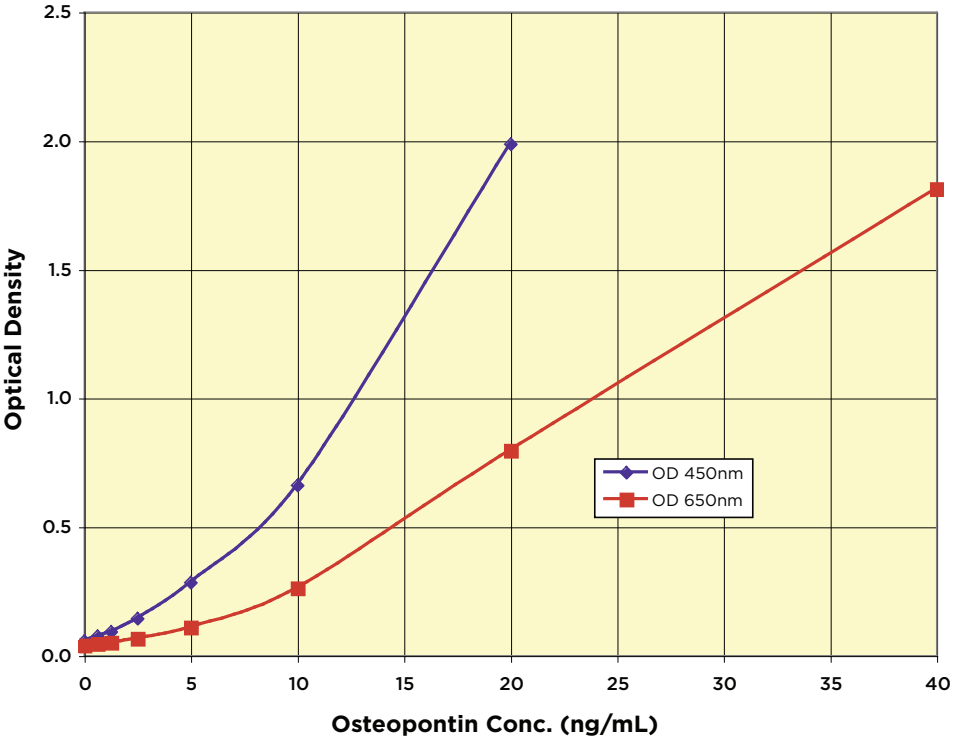
Typical Standard Curve



Always run your own standard curve for calculation of results.
Do not use this data.

Validation Data

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and standard #6. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.
Sensitivity was determined as 0.246 ng/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty replicates for each of the zero standard and a low concentration human urine sample.
Limit of Detection was determined as 0.248 ng/mL.

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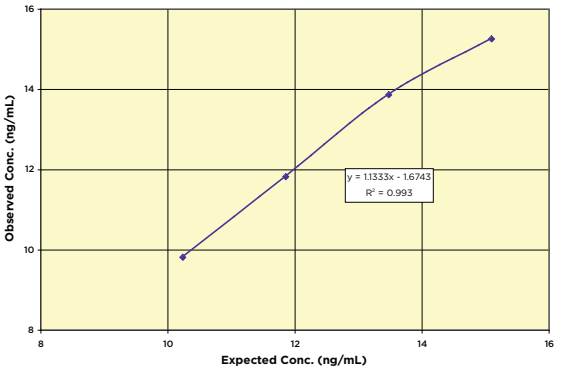
Linearity

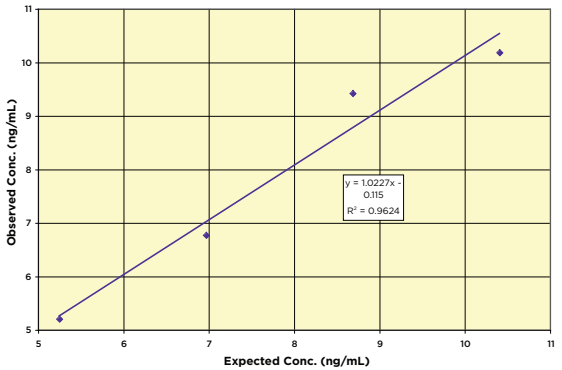
Linearity was determined by taking two human plasma samples diluted 1:40, one with a low diluted Osteopontin level of 8.62 ng/mL and one with a higher diluted level of 16.72 ng/mL and mixing them in the ratios given below. Milk linearity were determined by taking a sample diluted 1:3,000 with a value of 12.13 ng/mL and a sample diluted 1:10,000 with a value of 3.53 ng/mL and mixing in the ratios below. The measured concentrations were compared to the values previously determined.

High Sample	Low sample	Expected Conc. (ng/mL)		Observed Conc. (ng/mL)		% Recovery	
		Plasma	Milk	Plasma	Milk	Plasma	Milk
80%	20%	15.10	10.41	15.25	10.18	101.0	97.8%
60%	40%	13.48	8.69	13.86	9.42	102.8	108.4%
40%	60%	11.86	6.97	11.82	6.77	99.7	97.1%
20%	80%	10.24	5.25	9.81	5.20	95.8	99.0%
				Mean Recovery		**99.8%**	**100.6%**

Plasma Linearity



Milk Linearity



Intra Assay Precision

Three human samples, one urine, one milk and one EDTA plasma, were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Osteopontin concentrations were:

Sample	Osteopontin Conc. (ng/mL)	%CV
1	19.32	1.5
2	9.86	2.6
3	6.75	2.6

Inter Assay Precision

Three human samples, one urine, one milk and one EDTA plasma, were diluted with Assay Buffer and run in duplicates in ten assays run over multiple days by three operators. The mean and precision of the calculated Osteopontin concentrations were:

Sample	Osteopontin Conc. (ng/mL)	%CV
1	19.17	3.5
2	9.73	5.8
3	6.01	9.5

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Sample Values

Nine random plasma samples were tested in the assay. Adjusted values ranged from 179.9 to 746.0 ng/mL with an average of 386.7 ng/mL. Ten prostate cancer patient plasma samples were tested in the assay. Adjusted values ranged from 376.5 ng/mL to 2,271.5 ng/mL with an average value of 843.3 ng/mL.

Six random urine samples were tested in the assay. Adjusted values ranged from 404.3 to 4,595 ng/mL. When corrected for urine creatinine using the DetextX® Urinary Creatinine Detection kit, K002-H1, the values ranged from 646 to 33,491 ng/mg creatinine.

A clarified milk sample was also tested in the kit and its adjusted value was 36,480 ng/mL.

Cross Reactivity

Recombinant mouse OPN was tested in the kit. The reactivity was measured at 0.72%. Other species have not been tested in this kit.

This kit should only be used for human samples.

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Limited Warranty

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

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