

Datasheet for ABIN2815075

**Osteopontin ELISA Kit****3** Images[Go to Product page](#)

## Overview

Quantity: 96 tests

Target: Osteopontin (SPP1)

Reactivity: Human

Method Type: Sandwich ELISA

Application: ELISA

## Product Details

Purpose: The DetectX® Human Osteopontin kit is designed to quantitatively measure human Osteopontin present in biological samples and tissue culture media.

Brand: DetectX®

Sample Type: Plasma (EDTA), Urine, Milk, Tissue Culture Medium

Analytical Method: Quantitative

Detection Method: Colorimetric

Cross-Reactivity (Details): 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.

Components: Clear Coated 96 Well Plate One Plate Clear plastic microplate with break-apart strips coated with mouse anti-human Osteopontin.

Osteopontin Standard 2 Vials Two vials of recombinant human Osteopontin at 20 ng. Must be stored at -20°C

DetectX® Osteopontin Conjugate 5 mL A monoclonal antibody to human Osteopontin labeled with peroxidase.

## Product Details

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Assay Buffer Concentrate 28 mL A 5X concentrate that should be diluted with deionized or distilled water.

Wash Buffer Concentrate 30 mL A 20X concentrate that should be diluted with deionized or distilled water.

TMB Substrate 11 mL

Stop Solution 5 mL A 1N hydrochloric acid solution. Caustic.

Plate Sealer 1 each

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### Material not included:

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  $\mu$ L.

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.

## Target Details

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Target: Osteopontin (SPP1)

Alternative Name: Osteopontin (OPN) ([SPP1 Products](#))

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<sup>1</sup>. 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<sup>2</sup>, and it inhibits the ex-

## Target Details

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pression of inducible nitric oxide synthase (iNOS) in both macrophages and primary renal tubular epithelial cells to exert important protective effects on tissues<sup>3</sup>. It is also a key molecule in neo- plastic 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<sup>4</sup>. Plasma OPN has been shown to be a positive indicator of colon and lung cancers as well as metastatic carcinomas<sup>4-9</sup>. 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<sup>10,11</sup>. In addition, OPN inhibits inducible nitric oxide synthase activity, thereby protecting tumor cells from NO-mediated macrophage cytotoxic attack<sup>12</sup>. Recently it has been shown that OPN mRNA expression increases 37-40 fold in infarct tissue after a myocardial infarction<sup>13</sup>. 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<sup>14</sup>. OPN is found in atherosclerotic plaques and may drive a number of diabetic vascular pathologies<sup>15</sup>

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Pathways: [Regulation of Cell Size](#)

## Application Details

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Application Notes: 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.

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Comment: 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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Plate: Pre-coated

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## Application Details

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**Protocol:** 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.

**Reagent Preparation:** reagent PrePreparation 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	2	3	4	5	6	Assay Buffer Volume (µL)
Volume (µL)	250	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

**Sample Preparation:** EDTA plasma samples must be diluted  $\geq$  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  $\geq$  1:100. Urine samples must be diluted  $\geq$  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  $\geq 1:40$ . 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  $\geq 1:20$  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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### Assay Procedure:

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:

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	Mean OD Human Osteopontin Conc. (ng/mL)	(650nm)	(450 nm)	Alt. Standard
1.813	-	40	Standard 1	0.797
1.987	-	1.987		

## Application Details

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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. Typical Standard Curve # Optical Density # OD 450nm OD 650nm # # # # ! # " =absS]^]\bW\ 1]\Q \U [: Always run your own standard curve for calculation of results. Do not use this data.

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.

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.

Restrictions: For Research Use only

## Handling

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Precaution of Use: 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.

Storage: -20 °C, 4 °C, RT

Storage Comment: 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.

Images

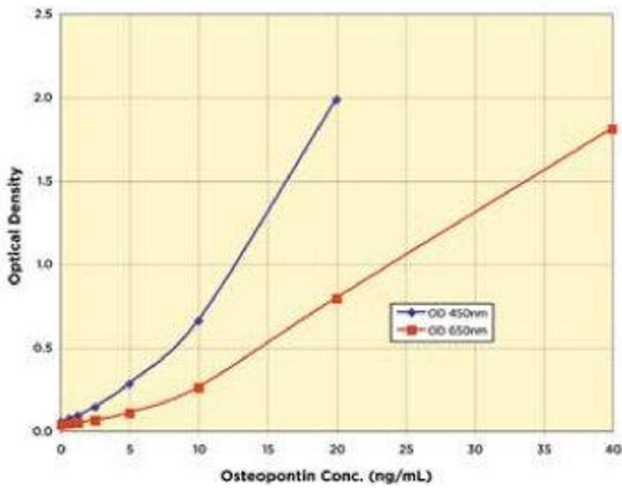


Image 1.

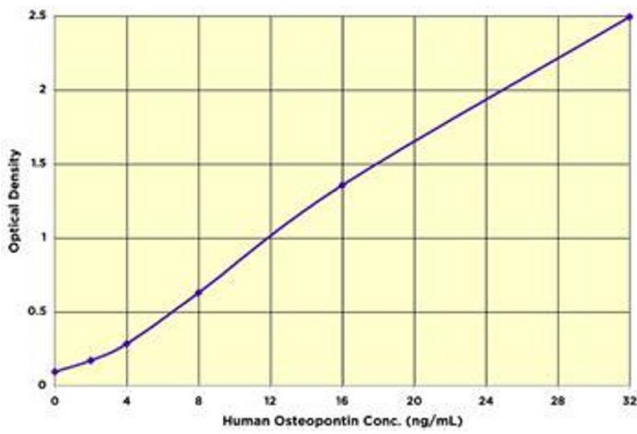


Image 2.

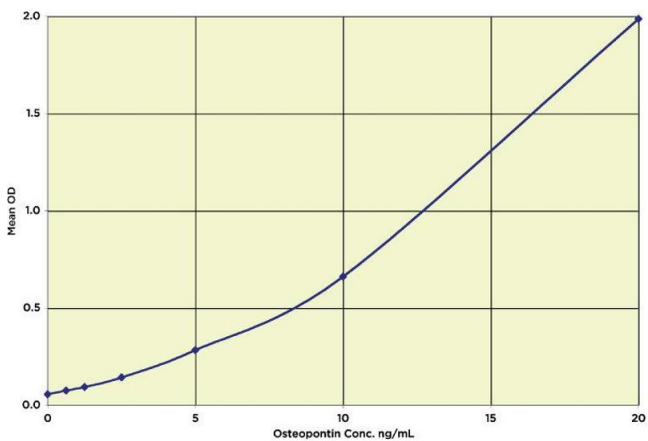


Image 3.