ARBOR ASSAYS™
Interactive Assay Solutions™

DetectX®

Human Osteopontin
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K021-H1

Updated Standard Range & Instruction

Sample Types Validated:

EDTA Plasma, Urine, Milk and Tissue Culture Media

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

TABLE OF CONTENTS

BACKGROUND

Osteopontin (OPN) is vital in mineralization, blood vessel formation, cell survival, inflammation, and inhibition of iNOS expression, which protects tumor cells from NO-mediated macrophage cytotoxic attack[1]. It is also a key molecule in neoplastic transformation and cancer development in various tumors, playing a critical role in invasiveness, progression, and metastasis[2]. Plasma OPN is a positive indicator of colon and lung cancers and metastatic carcinomas[2-9]. In addition, OPN mRNA expression increases approximately 40-fold in infarct tissue after myocardial infarction[10].

1. A. O'Regan, J.S. Berman, Int J Exp Pathol (2000) 81:373–90.
2. D.T. Denhardt, et al., J. Clin. Invest., (2001) 107: 1055-1061.
3. D. Agrawal, et al., J. Natl. Cancer Inst., (2002) 94: 513-521.
4. S.B. Hotte, et al., Cancer, (2002) 95: 506-512.
5. K.A. Furger, et al., Curr. Mol. Med., (2001) 1: 621-632.
6. H. Singhal, et al., Clin. Cancer Res., (1997) 3: 605-611.
7. A.F. Chambers, H. Singhal, et al., Lung Cancer, (1996) 15: 311-323.
8. L.F. Brown et al., Am. J. Pathol., (1994) 145: 610-623.
9. D. Coppola, et al., Clin. Cancer Res., (2004) 10: 184-190.
10. N.A. Trueblood, et al., Circ Res (2001) 88:1080–7.

ASSAY PRINCIPLE

The DetectX® Human Osteopontin Immunoassay Kit is designed to quantitatively measure human Osteopontin present in biological samples and tissue culture media. 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	Catalog No.
Allopregnanolone ELISA Kits	K061-H1/H5
Arg^{8} - Vasopressin (AVP) ELISA Kits	K049-H1/H5
Atrial Natriuretic Peptide (ANP) ELISA Kits	K071-H1/H5
C-Reactive Protein (CRP) Human ELISA Kits	K069-H1/H5
Cyclic AMP Direct Chemiluminescent ELISA Kits	K019-C1/C5
Cyclic AMP Direct ELISA Kits	K019-H1/H5
Histone Demethylase Activity Kit	K010-F1
PKA (Protein Kinase A) Colorimetric Activity Kit	K027-H1
Urinary Creatinine Detection Kits	K002-H1/H5

SUPPLIED COMPONENTS

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

Osteopontin Standard
Two vials of recombinant human Osteopontin at 20 ng. **MUST BE STORED AT -20°C.**
2 Vials — Catalog Number C077-1EA

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

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

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

TMB Substrate
11 mL — Catalog Number X019-11ML

Stop Solution
A 1M hydrochloric acid solution. **CAUSTIC**.
5 mL — Catalog Number X020-5ML

Plate Sealer
1 Each — Catalog Number X002-1EA

STORAGE INSTRUCTIONS

The unopened kit must be stored at -20°C.

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

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Polypropylene test tubes. **Note**: The use of glass test tubes reduces the observed signal by approximately 25%.

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 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.

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.

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

Plasma Samples

EDTA plasma samples must be diluted ≥ 1:20 with 1X 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

Urine samples must be diluted ≥ 1:10 with 1X 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. For comparison to creatinine as a urine volume marker, please see our NIST-calibrated Urinary Creatinine Detection Kits, K002-H1/H5.

Milk Samples

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 1X 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.

Tissue Culture Media

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

Any samples with Osteopontin concentrations outside the standard curve range should be diluted further with 1X 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.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

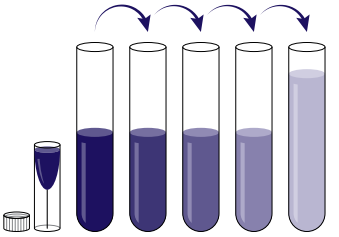
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. The 1X Assay Buffer is stable at 4°C for 3 months.

Wash Buffer

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

Standard Preparation

Add 1000 µL of 1X Assay Buffer to one vial of OPN standard to generate Standard 1 at 20 ng/mL. Vortex gently, let sit for 5 minutes, and vortex gently again. Label test tubes as #2 through #7. Pipet 250 µL of 1X Assay Buffer into tubes #2 to #7. Add 250 µL of the 20 ng/mL Osteopontin Standard solution to tube #2 and vortex completely. Take 250 µL of the Osteopontin solution in tube #2 and add it to tube #3 and vortex completely. Repeat the serial dilutions for tubes #4 through #7. The concentration of Osteopontin in the vial and tubes #1 through #7 will be 20, 10, 5, 2.5, 1.25, 0.625, and 0.313 ng/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
1X Assay Buffer Volume (µL)		250	250	250	250	250	250
Addition	Vial	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	1000	250	250	250	250	250	250
Final Conc (ng/mL)	20	10	5	2.5	1.25	0.625	0.313

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Osteopontin concentrations.

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 1X Assay Buffer into the zero standard wells.
3. Seal the plate if desired and incubate at room temperature for 60 minutes. Aspirate the plate and wash each well 4 times with 300 µL 1X 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. Reseal the plate and incubate at room temperature for 60 minutes.
6. Aspirate the plate and wash each well 4 times with 300 µL 1X 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.
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.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

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.

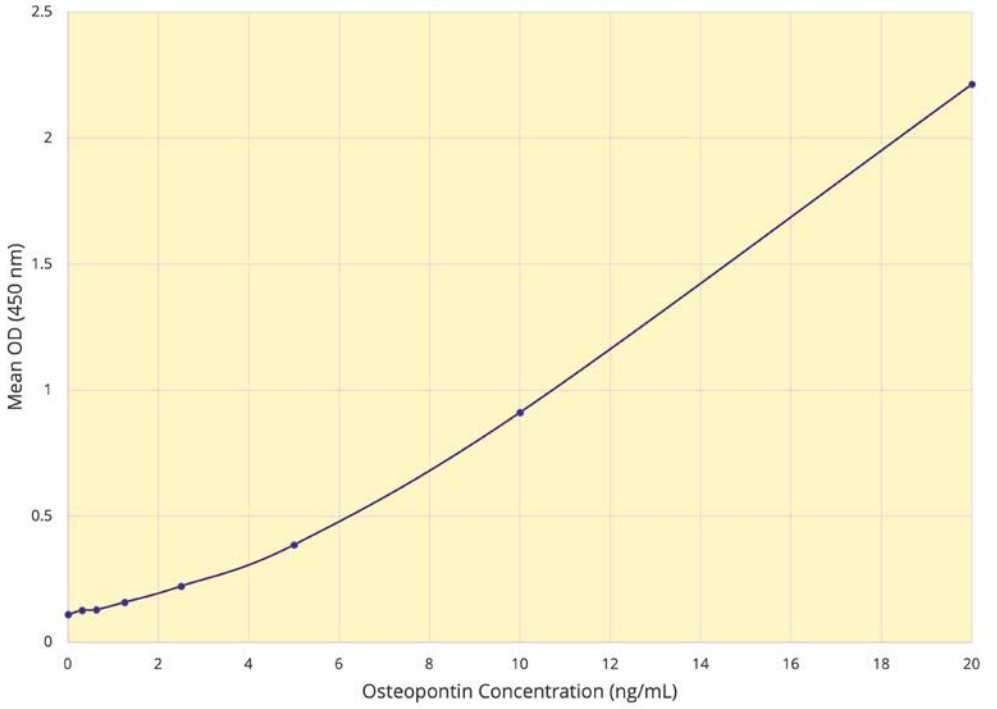
Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-osteopontin-human-(opn)-eia-kit.assay

TYPICAL DATA

Sample	Mean OD (450 nm)	Human Osteopontin Conc. (ng/mL)
Standard 1	2.213	20
Standard 2	0.911	10
Standard 3	0.386	5
Standard 4	0.222	2.5
Standard 5	0.158	1.25
Standard 6	0.129	0.625
Standard 7	0.126	0.313
Zero	0.109	0
Sample 1	1.095	11.6
Sample 2	0.701	8.1

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

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 #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.151 ng/mL**.

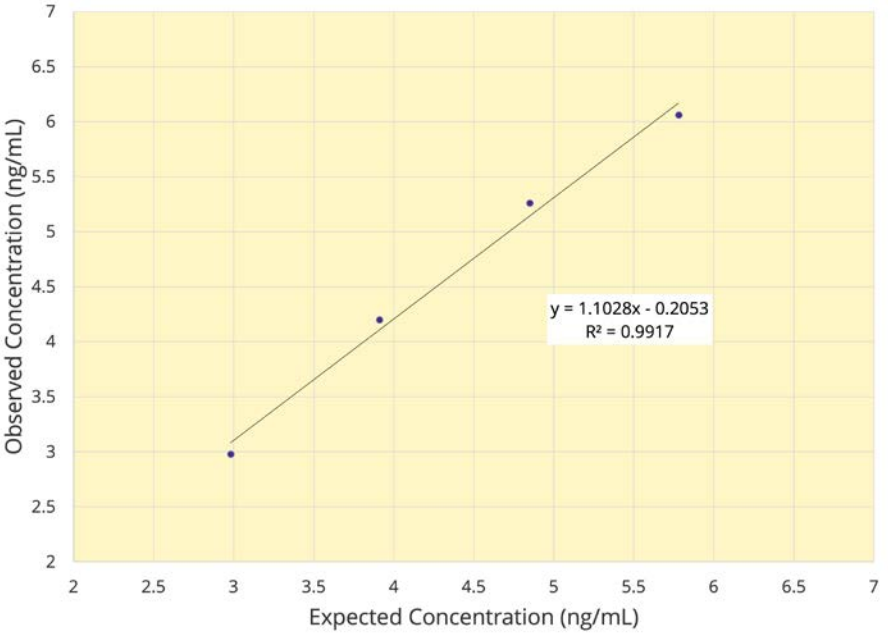
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty replicates for each of the zero standard and a low concentration milk sample. **Limit of Detection was determined as 0.303 ng/mL**.

Linearity

Linearity was determined by taking two diluted plasma samples, one with an Osteopontin level of 1.96 ng/mL and one with a level of 7.54 ng/mL and mixing them in the ratios given below. The measured concentrations were compared to the values previously determined.

High Sample	Low sample	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	3.08	3.18	103.2%
60%	40%	4.19	4.55	108.6%
40%	60%	5.31	5.54	104.3%
20%	80%	6.42	6.50	101.2%
			Mean Recovery	**104.4%**

Plasma Linearity



Intra Assay Precision

Three samples, one urine, one milk and one EDTA plasma, were diluted with 1X Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Osteopontin concentrations were:

Sample	Osteopontin Concentration (ng/mL)	%CV
1	11.49	3.0
2	8.13	2.5
3	3.16	3.7

Inter Assay Precision

Three samples, one urine, one milk and one EDTA plasma, were diluted with Assay Buffer and run in duplicates in 20 assays run over multiple days by 5 operators. The mean and precision of the calculated Osteopontin concentrations were:

Sample	Osteopontin Concentration (ng/mL)	%CV
1	11.83	4.2
2	8.39	3.0
3	3.10	7.7

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.

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.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

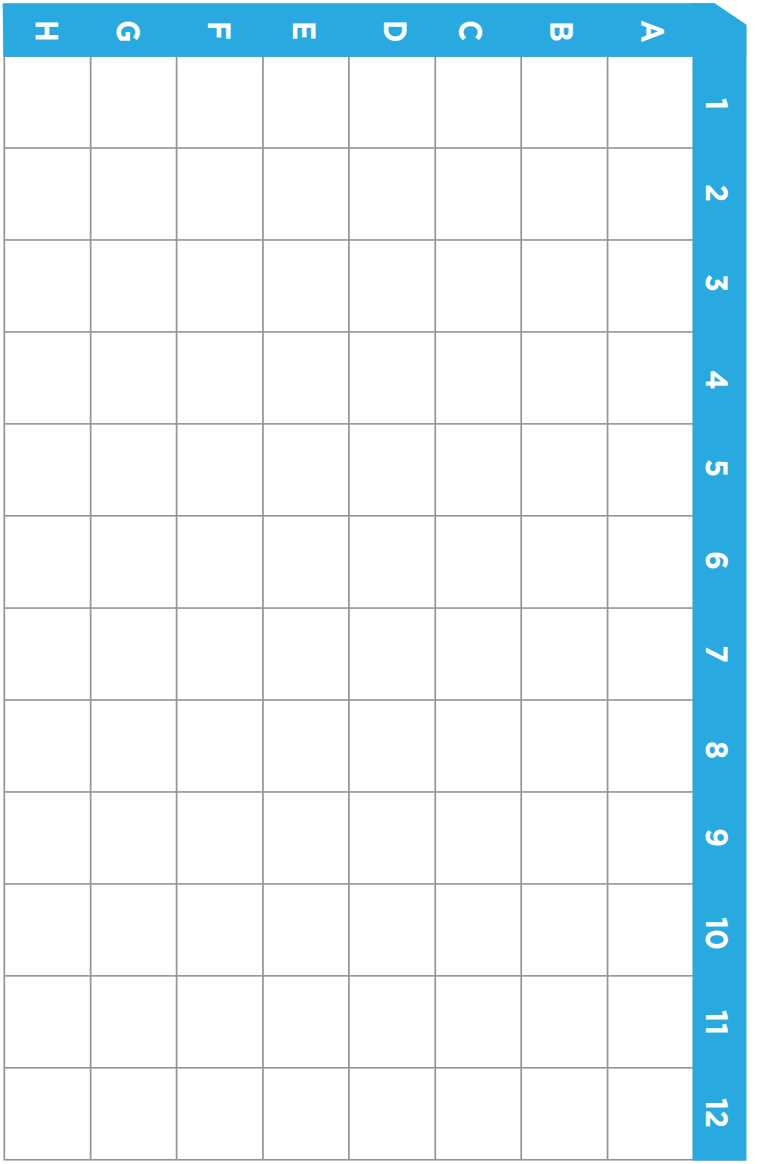
Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.



Printed on Forest Stewardship Council certified paper