

## ab269374 – Human Osteopontin SimpleStep ELISA® Kit

For the quantitative measurement of Osteopontin in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, urine, milk, and CSF.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: [www.abcam.com/ab269374](http://www.abcam.com/ab269374)

**Storage and Stability:** Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

### Materials Supplied

Item	Quantity	Storage Condition
Human Osteopontin Capture Antibody 10X	600 µL	+4°C
Human Osteopontin Detector Antibody 10X	600 µL	+4°C
Human Osteopontin Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	+4°C
Plate Seal	1	+4°C

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.  
Deionized water.  
Multi- and single-channel pipettes.  
Tubes for standard dilution.  
Plate shaker for all incubation steps.  
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

### Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

**1X Wash Buffer PT:** Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

**Antibody Cocktail:** Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

### Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the Osteopontin standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 100 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1–8.
3. Add 372 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	<b>Stock Standard</b>	28	372	100,000	7,000
2	Standard#1	150	150	7,000	3,500
3	Standard#2	150	150	3,500	1,750
4	Standard#3	150	150	1,750	875
5	Standard#4	150	150	875	437.50
6	Standard#5	150	150	437.50	218.75
7	Standard#6	150	150	218.75	109.38
8	Blank Control	0	150	N/A	N/A

## Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	3.13 - 50%
Plasma - Heparin	1.56 - 12.5%
Plasma - EDTA	1.56 - 12.5%
Plasma - Citrate	3.13 - 12.5%
Stimulated PBMC Cell Culture Supernatant	3.13 - 50%
Urine	1:6,400 - 1:400
Human Milk	1:320,000 - 1:20,000
Cerebrospinal Fluid	0.08 - 2.5%

**Serum** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

**Plasma** Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:8 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Cell Culture Supernatants** Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

**Cerebrospinal Fluid (CSF)** Dilute cerebrospinal fluid at least 1:32 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

**Urine** Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:400 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

**Milk** De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute the de-fatted milk samples at least 1:20,000 into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

## Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Differences in well absorbance or "edge effects" have not been observed with this assay.

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## Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

We recommend that you assay all standards, controls and samples in duplicate

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

*Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.*

**Note:** The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

**Note** that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

**Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:**

[www.abcam.com/protocols/the-complete-elisa-guide](http://www.abcam.com/protocols/the-complete-elisa-guide)

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### Additional information

#### ASSAY SPECIFICITY

This kit is designed for the quantification of human Osteopontin.

The standard protein in this kit is an N-terminal fragment, representing approximately 93% of the mature full-length mass calibrated to full-length human Osteopontin.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, urine, milk, and CSF.

Saliva, cell extract, and tissue extract samples have not been tested with this kit.

#### SPECIES REACTIVITY

Other species reactivity was determined by measuring 1:2 serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Monkey was found to have 13% cross-reactivity.

Reactivity < 5% was determined for the following species: Mouse, Rat, Cow

Other species reactivity not determined.

#### CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.  
 $\Delta$  Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

#### TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.073	0.075	0.074
109.38	0.110	0.111	0.110
218.75	0.169	0.171	0.170
437.50	0.288	0.297	0.293
875	0.511	0.552	0.532
1,750	0.945	0.989	0.967
3,500	1.761	1.835	1.798
7,000	3.111	3.150	3.131

Table 1. Example of human Osteopontin standard curve in Sample Diluent NS. The Osteopontin standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

#### TYPICAL SAMPLE VALUES

##### Sensitivity:

The calculated minimal detectable dose (MDD) is 88 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=20) and adding 2 standard deviations then extrapolating the corresponding concentration.

##### Recovery

Three concentrations of Osteopontin were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
5% Serum	102	99 – 104
5% Plasma – Citrate	92	91 – 93
5% Plasma – EDTA	99	95 – 102
5% Plasma – Heparin	93	90 – 96
1:400 Urine	98	96 – 100
1:100,000 Human Milk	96	93 – 98
50% Stimulated PBMC Cell Culture Supernatant	108	102 – 115
0.5% Cerebrospinal Fluid	86	82-93

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Osteopontin was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Serum	12.5% Human Plasma (Citrate)	12.5% Human Plasma (EDTA)	12.5% Human Plasma (Heparin)
Undiluted	pg/mL	7,688	1,564	1,477	2,376
	% Expected value	100	100	100	100
2	pg/mL	3,591	846	819	1,247
	% Expected value	93	108	1111	105
4	pg/mL	2,016	456	424	638
	% Expected value	105	117	115	107
8	pg/mL	1,071	NL	211	313
	% Expected value	111	NL	114	106
16	pg/mL	560	NL	NL	NL
	% Expected value	116	NL	NL	NL

NL – Non-Linear

Dilution Factor	Interpolated value	1:400 Human Urine	1:20,000 Human Milk	50% Stimulated PBMC Supernatant	2.5% Cerebro-spinal Fluid
Undiluted	pg/mL	3,780	4,334	2,159	3517
	% Expected value	100	100	100	100
2	pg/mL	1,843	2,146	985	1791
	% Expected value	98	99	91	102
4	pg/mL	968	1,112	486	890
	% Expected value	102	103	90	101
8	pg/mL	468	572	263	454
	% Expected value	99	105	97	103
16	pg/mL	242	266	145	243
	% Expected value	102	98	107	111

Precision

Mean coefficient of variations of interpolated values of Osteopontin from one concentration of serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	3	8
CV (%)	2.1	2.8

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

[www.abcam.com/protocols/the-complete-elisa-guide](http://www.abcam.com/protocols/the-complete-elisa-guide)

Technical Support

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