

# Human Osteopontin

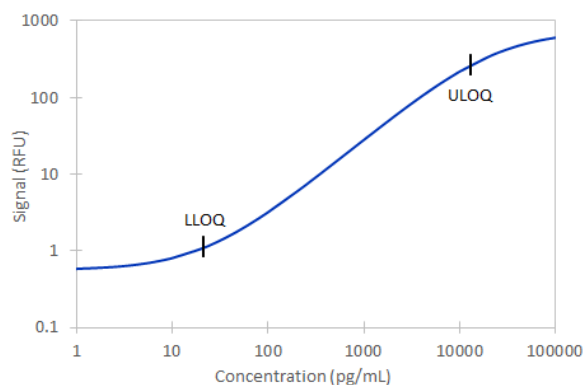
## Product Description

Simple Plex™ assay is for the detection of human Osteopontin (OPN) in cell culture supernatant (CCS), serum, plasma (EDTA/Heparin), urine, and cerebrospinal fluid (CSF) in Sample Diluent SD13.

## Calibration Curve

The factory generated calibration curve shown below was compiled by averaging replicates of each calibrator from multiple runs. The 4PL curve fit shows calibrator concentration as a function of signal intensity (relative fluorescent units, RFU).

Human serum and plasma assays are quality control tested using a serum/plasma based Bio-Techne Reference Material. Curves may be adjusted to this material for lot-to-lot consistency.



## Limits of Quantification

Data shown represents typical performance results of the Simple Plex Human OPN Assay.

	Concentration (pg/mL)
Limit of Detection (LOD)	7.33
Lower Limit of Quantitation (LLOQ)	21.0
Upper limit of Quantitation (ULOQ)	12,800

LOD is calculated by adding three standard deviations to the mean background signal determined from multiple runs.

LLOQ and ULOQ are calculated across multiple cartridge lots and Ellas as the in-well concentration range in which curve points recover 80-120% with a coefficient of variation (CV) of less than 20%, and in which measured samples recover 80-120% upon serial dilution with a CV less than 15%.

## Endogenous Levels

Endogenous levels were calculated from multiple samples. Samples were from apparently healthy volunteers. No medical histories were available for the donors in this study. The values provided below are for informational purposes only. Actual concentration values and detectability rates may vary across different sample cohorts.

Sample Type	Mean (pg/mL)	Range (pg/mL)	% above LLOQ
Serum (n=10)	41,900	24,200 - 63,800	100
EDTA plasma (n=10)	76,620	57,200 - 94,100	100
Heparin plasma (n=10)	73,540	53,300 - 98,100	100
Urine (n=10)	1,696,000	494,000 - 2,962,000	100
CSF (n=11)	196,200	27,270 - 488,411	100

## Precision

**Intra-Assay Precision:** Multiple replicates of each control were tested in one assay.

**Inter-Assay Precision:** Replicates of each control were tested in multiple runs performed by at least three technicians using two lots of reagents.

Parameter	Intra-Assay		Inter-Assay	
	Low	High	Low	High
n	16	16	42	42
Mean (pg/mL)	221	10,403	93.2	4970
Standard Deviation	16.2	661	9.46	454
CV (%)	7.3	6.4	10.1	9.5

## Recovery

Recovery was not necessary as OPN shows 5 points of natural linearity.

## Linearity

Samples containing and/or spiked with high concentrations of human OPN were serially diluted with Sample Diluent to produce samples within the dynamic range of the assay.

Dilution	Parameter	CGS (n=4)	Serum (n=4)	EDTA plasma (n=4)
1:2	Avg % of Expected	98	103	100
	Range (%)	91-104	94-116	92-104
1:4	Avg % of Expected	93	101	101
	Range (%)	87-102	93-113	95-112
1:8	Avg % of Expected	88	106	100
	Range (%)	81-99	89-120	91-111
1:16	Avg % of Expected	90	107	101
	Range (%)	73-111	97-123	78-111

Dilution	Parameter	Heparin plasma (n=4)	Urine (n=9)	CSF (n=7)
1:2	Avg % of Expected	98	103	100
	Range (%)	91-104	94-116	92-104
1:4	Avg % of Expected	93	101	101
	Range (%)	87-102	93-113	95-112
1:8	Avg % of Expected	88	106	100
	Range (%)	81-99	89-120	91-111
1:16	Avg % of Expected	90	107	101
	Range (%)	73-111	97-123	78-111

## Specificity

This assay recognizes natural and recombinant human OPN. The factors listed were prepared at 320 ng/mL in Sample Diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a recombinant human OPN control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

- CD44
- Enterokinase
- FAM20A
- FAM20C
- Fibronectin
- IGFBP-5
- IL-6
- Integrin  $\alpha$ 5
- Integrin  $\alpha$ 9/ Integrin  $\beta$ 1 complex
- MMP-3
- MMP-7
- Prolactin
- Serum Albumin
- Thrombin
- TIMP-1

## Sample Collection and Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Cell Culture Supernatant:** Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Serum:** Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Cerebrospinal Fluid:** Freeze sample within one hour of collection to avoid aggregation. Store samples at  $\leq -70$  °C.

**Urine:** Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately, or aliquot and store at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Note:** Grossly hemolyzed or icteric samples are not suitable for use with this assay.

## Sample Preparation

CCS samples require a minimum 5-fold dilution with Sample Diluent. A suggested 5-fold dilution can be achieved by adding 20  $\mu$ L of sample to 80  $\mu$ L of Sample Diluent. Samples above the ULOQ require further dilution.

Serum and plasma samples require a minimum 100-fold dilution with Sample Diluent. A suggested 100-fold dilution can be achieved by adding 10  $\mu$ L of sample to 90  $\mu$ L of Sample Diluent (Dilution 1). Complete the 100-fold dilution by adding 10  $\mu$ L of Dilution 1 to 90  $\mu$ L of Sample Diluent. Samples above the ULOQ require further dilution.

Urine require a minimum 2000-fold dilution with Sample Diluent. A suggested 2000-fold dilution can be achieved by adding 10  $\mu$ L of sample to 90  $\mu$ L of Sample Diluent (Dilution 1). Then add 10  $\mu$ L of Dilution 1 to 90  $\mu$ L of Sample Diluent (Dilution 2). Complete the 2000-fold dilution by adding 10  $\mu$ L of Dilution 2 to 190  $\mu$ L of Sample Diluent. Samples above the ULOQ require further dilution.

## Assay Protocol

Refer to the Product Insert and CoA provided on our website for the specific assay protocol and precautions. A generic product protocol can be found online at: [www.bio-techne.com/simple-plex-protocol](http://www.bio-techne.com/simple-plex-protocol)

Download the CoA and product insert:  
[www.bio-techne.com/resources/cofa-finder-tool](http://www.bio-techne.com/resources/cofa-finder-tool)