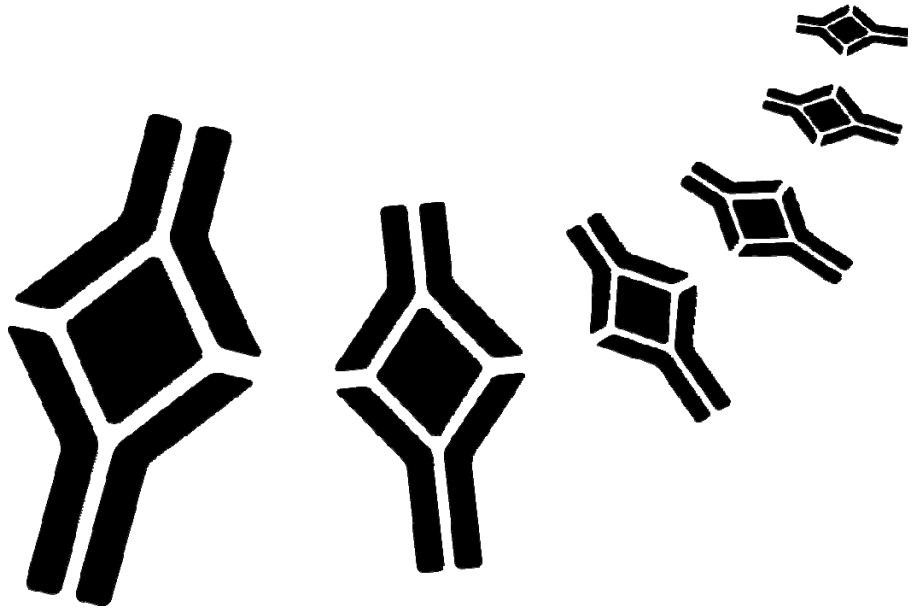
BioVendor
Research
and Diagnostic Products



HUMAN OSTEOPONTIN (OPN) ELISA

Product Data Sheet

Cat. No.: BBT0482R

For Research Use Only

VERSION 51 240310 03

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

For quantitative detection of human OPN in sera, plasma, urine, body fluids, tissue lysates or cell culture supernates.

2. STORAGE, EXPIRATION

Storage
Store at 4°C for frequent use, at -20°C for infrequent use.
Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration
Four months at 4°C and eight months at -20°C.

3. INTRODUCTION

Osteopontin (OPN) also called urinary stone protein, secreted phosphoprotein 1 (SPP1), bone sialoprotein, and early T lymphocyte activation 1 (ETA1). Osteopontin is a phosphorylated glycoprotein secreted to the mineralizing extrOPNllular matrix by osteoblasts during bone development. It is believed to facilitate the attachment of osteoblasts and osteoclasts to the extrOPNllular matrix, allowing them to perform their respective functions during osteogenesis.[1] Osteopontin is presumably involved in stone formation as stone matrix.[2] The deduced protein sequence reveals a 317-amino acid protein (34,982 Da) containing a 16-amino acid hydrophobic signal sequence and a 33,352-Da protein destines to undergo extensive post-translational modifications before being secreted from the cell. The gene is located on human chromosome 4.[3] The standard product used in this kit is recombinant human OPN, consisting of 17-314 amino acid sequence with the molecular mass of 32.9KDa. As a result of glycosylation, the molecular mass is 60-65KDa.

4. TEST PRINCIPLE

BioVendorr's human OPN ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human OPN specific-specific polyclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human OPN amount of sample captured in plate.

5. PRECAUTIONS

Notice for Application of Kit

- Before using Kit, spin tubes and bring down all components to bottom of tube.
- Duplicate well assay was recommended for both standard and sample testing.
- Don't let 96-well plate dry, dry plate will inactivate active components on plate.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

6. REAGENT SUPPLIED

- Lyophilized recombinant human OPN standard: 10 ng/tube×2.
- One 96-well plate precoated with anti- human OPN antibody.
- Sample diluent buffer: 30 ml
- Biotinylated anti- human OPN antibody : 130 µl, dilution 1:100.
- Antibody diluent buffer: 12 ml.
- Avidin-Biotin-Peroxidase Complex (ABC) : 130 µl, dilution 1:100.
- ABC diluent buffer: 12 ml.
- TMB color developing agent: 10 ml.
- TMB stop solution: 10 ml.

7. MATERIAL REQUIRED BUT NOT SUPPLIED

Microplate reader in standard size.

- Automated plate washer.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- Clean tubes and Eppendorf tubes.
- Washing buffer (neutral PBS or TBS).
- Preparation of 0.01M **TBS:** Add 1.2 g Tris, 8.5 g Nacl; 450 µl of purified acetic acid or 700 µl of concentrated hydrochloric acid to 1000 ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
- Preparation of 0.01 M **PBS:** Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

8. PREPARATION OF REAGENTS AND SAMPLES

Plate Washing

Aspirate the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes.

Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.

Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C.

Avoid repeated freeze-thaw cycles.

Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C

Serum: Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C. EDTA and citrate are not recommended as the anticoagulant.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice.

Serum/ plasma samples

Dilute samples 10x with Sample dilution buffer just prior to the assay, e.g. 20 μl of sample + 180 μl of Sample dilution buffer for singlets, or preferably 30 μl of sample + 270 μl of Sample dilution buffer for duplicates.

Urine samples

Dilute samples 200x with Sample dilution buffer just prior to the assay in two steps as follows:

Dilution A (10x):

Add 5 μl of sample into 45 μl of Sample dilution buffer. Mix well (not to foam). Vortex is recommended.

Dilution B (20x):

Add 7 μl of Dilution A into 133 μl of Sample dilution buffer for singlets, or preferably 13 μl of Dilution A into 247 μl of Sample dilution buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Stability and storage:

Serum/plasma samples should be stored at -20°C, or preferably at -70°C for long-term storage. Urine samples have to be stored at -70°C. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

The sample must be well mixed with the diluents buffer.

Other type of samples:

High target protein concentration (100-1000 ng/ml). The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.

Medium target protein concentration (10-100 ng/ml). The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.

Low target protein concentration (156-10,000 pg/ml). The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.

Very Low target protein concentration (≤156 pg/ml). No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

A. Reconstitution of the human OPN standard: OPN standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of OPN standard (10ng per tube) are included in each kit. Use one tube for each experiment.
   1 10,000 pg/ml of human OPN standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
   2 5000 pg/ml→156 pg/ml of human OPN standard solutions: Label 6 Eppendorf tubes with 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml,

respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10, 000 pg/ml OPN standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human OPN antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
   1 The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
   2 Biotinylated anti-human OPN antibody should be diluted in 1:99 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
   1 The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
   2 Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:99 with the ABC dilution buffer and mixed thoroughly.

Ask for protocol at info@biovendor.com if assaying tissue culture medium samples.

9. ASSAY PROCEDURE

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard OPN detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of OPN amount in samples.

1. Aliquot 0.1ml per well of the 10,000 pg/ml, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml human OPN standard solutions into the precoated 96-well plate. Add 0.1 ml of the sample diluent buffer into the control well (Zero well). Add 0.1 ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each human OPN standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, aspirate plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human OPN antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Aspirate the washing buffer and blot the plate onto paper towels or other absorbent material.

6. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Aspirate the washing buffer and blot the plate onto paper towels or other absorbent material.
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C for 16-20 min (shades of blue can be seen in the wells with the four most concentrated human OPN standard solutions; the other wells show no obvious color).
9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{.450}$) = (the $O.D._{.450}$ of each well) – (the $O.D._{.450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{.450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human OPN concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

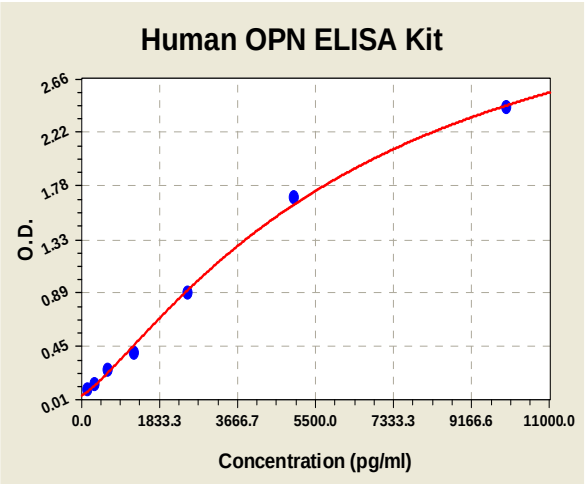
1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C for 16-20 min.
5. Add TMB stop solution and read.

10. CALCULATIONS

Typical Data Obtained from Human OPN

Concentration	0.0 pg/ml	156 pg/ml	313 pg/ml	625 pg/ml	1250 pg/ml	2500 pg/ml	5000 pg/ml	10,000 pg/ml
O.D	0.056	0.098	0.137	0.264	0.397	0.895	1.682	2.423

Typical Human OPN ELISA Kit Standard Curve
This standard curve was generated at Biovendor for demonstration purpose only. A standard curve must be run with each assay.



11. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human OPN ELISA are presented in this chapter.

- **Sensitivity**

< 50 pg/ml

- **Specificity**

No detectable cross-reactivity with any other cytokine.

- **Range**

156 pg/ml -10,000 pg/ml

- **Precision**

Intra-assay (Within-Run, n=8)

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
1	8459	659	7.8
2	2818	265	9.4

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	*Observed (pg/ml)*	*Expected (pg/ml)*	*Recovery O/E (%)*
1	- 2x	5466 2840	- 2733	- 103.9
2	- 2x	7885 4303	- 3942	- 109.1
3	- 2x	6605 3207	- 3302	- 101.7

12. REFERENCES

References to human OPN:

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2. Kohri, K.; Suzuki, Y.; Yoshida, K.; Yamamoto, K.; Amasaki, N.; Yamate, T.; Umekawa, T.; Iguchi, M.; Sinohara, H.; Kurita, T. Molecular cloning and sequencing of cDNA encoding urinary stone protein, which is identical to osteopontin. *Biochem. Biophys. Res. Commun.* 184: 859-864, 1992.
3. Fisher, L. W.; McBride, O. W.; Termine, J. D.; Young, M. F. Human bone sialoprotein: deduced protein sequence and chromosomal localization. *J. Biol. Chem.* 265: 2347-2351, 1990.

For more references on this product see our WebPages at www.biovendor.com

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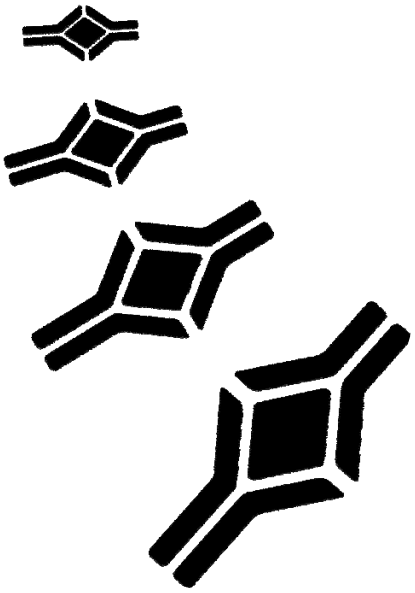

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