Datasheet for ABIN625068

Osteopontin ELISA Kit

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Overview

Quantity:	96 tests
Target:	Osteopontin (SPP1)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	50-18000 pg/mL
Minimum Detection Limit:	50 pg/mL
Application:	ELISA

Product Details

Purpose:	Human Osteopontin (SPP1) ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with any of the following cytokines tested (human Angiogenin, BDNF, BLC, ENA-78, FGF- 4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.
Sensitivity:	< 50 pg/mL

Product Details

Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	Osteopontin (SPP1)
Alternative Name:	Osteopontin (SPP1 Products)
Background:	Osteopontin (OPN) is an extracellular matrix cell adhesion protein which is abundant in bone and which is synthesized by preosteoblasts, osteoblasts and osteoclastic cells that are localized in the mineralized phase of bone matrix. It is an acidic, phosphorylated, sialic acid-rich Ca2+ binding protein. Osteopontin contains a signal sequence and is a secreted protein. It is involved in recruiting and stimulating macrophages and lymphocytes as part of a nonspecific response to microbial infections. Murine macrophages cell lines and resident macrophages show various levels of expression of the osteopontin gene, which can be enhanced by a variety of macrophage stimulating agents. The Human OPN ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human OPN in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human OPN coated on a 96-well plate. Standards and samples are pipetted into the wells and OPN present in a sample is bound to the wells by the immobilized

Target Details

	antibody. The wells are washed and biotinylated anti-human OPN antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of OPN bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	6696
UniProt:	P10451
Pathways:	Regulation of Cell Size

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples3 - 100 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) is used for dilution of culture supernatants and urine. Suggested dilution for normal serum/plasma: 3-100 fold*. * Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water. 4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µL Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine, Assay

	Diluent B should be diluted 5-fold with deionized or distilled water) into Item C vial to prepare a 100 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 90 µL OPN standard from the vial of Item C, into a tube with 410 µL Assay Diluent A or 1x Assay Diluent B to prepare a 18,000 pg/mL stock standard solution. Pipette 400 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL). 200 µL 200myl 200 µL 200 µL 200 µL 90 µL standard + 410 µL 18000 6000 2000 666.7 222.2 74.07 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP- Streptavidin concentrate should be diluted 500-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 20 µL of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent B to prepare a 500-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution (200 µL each). 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with shaking . 5. Discard the solution and wash 4 times with 1x Wash Solution (200 µL each). 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with shaking. 7. Discard the solution and wash 5 times with 1x Wash Solution (200 µL each). 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with shaking.

Application Details

	9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Human OPN concentration (pg/mL) O D =4 50 n m 0.1 1 10 10 100 1,000 10,000 100,000 Assay Diluent A Human OPN concentration (pg/mL) O D =4 50 n m 0.1 1 10 10 100 1,000 10,000 100,000 Assay Diluent B Sensitivity: The minimum detectable dose of OPN is typically less than 50 pg/mL. Recovery: Recovery was determined by spiking various levels of human OPN into human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 89.92 88-108 Plasma 92.41 90-109 Cell culture media 103.5 95-118 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 87 92 102 Range (%) 86-108 88-109 93-115 1:4 Average % of Expected 92 90 97 Range (%) 88-107 87-108 91-111 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Hussein, Alhazmi, Alzahrani, El-Askary, Alghamdy, Bayomy, Selim, Alghamdy: "Osteopontin as a marker for response to pegylated interferon Alpha-2b treatment in Chronic HCV Saudi patients." in: **African health sciences**, Vol. 17, Issue 2, pp. 366-372, (2018) (PubMed).

Publications

Wu, Ding, Han, Arriens, Wei, Han, Pedroza, Jiang, Anolik, Petri, Sanz, Saxena, Mohan: "Antibody-Array-Based Proteomic Screening of Serum Markers in Systemic Lupus Erythematosus: A Discovery Study." in: **Journal of proteome research**, Vol. 15, Issue 7, pp. 2102-14, (2016) (PubMed).

Filis, Martinakis, Galyfos, Sigala, Theodorou, Andreadou, Zografos: "Osteopontin and Osteoprotegerin as Potential Biomarkers in Abdominal Aortic Aneurysm before and after Treatment." in: **International scholarly research notices**, Vol. 2014, pp. 461239, (2016) (PubMed).

Gluba-Brzózka, Michalska-Kasiczak, Franczyk-Skóra, Nocu?, Banach, Rysz: "Markers of increased cardiovascular risk in patients with chronic kidney disease." in: **Lipids in health and disease**, Vol. 13, pp. 135, (2014) (PubMed).

Shaker, El-Shehaby, Fayez, Zahra, Marzouk, El Raziky: "Osteopontin gene polymorphisms as predictors for the efficacy of interferon therapy in chronic hepatitis C Egyptian patients with genotype 4." in: **Cell biochemistry and function**, Vol. 31, Issue 7, pp. 620-5, (2014) (PubMed).

There are more publications referencing this product on: Product page

Images

ELISA

Image 1.