Datasheet for ABIN625067

Osteoprotegerin ELISA Kit

1 Image | 23 Publications

Go to Product page

Overview

Quantity:	96 tests
Target:	Osteoprotegerin (TNFRSF11B)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	1-900 pg/mL
Minimum Detection Limit:	1 pg/mL
Application:	ELISA

Product Details

Purpose:	Human Osteoprotegerin (TNFRSF11B) 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 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-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin (OB), 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:	< 1 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:	Osteoprotegerin (TNFRSF11B)
Alternative Name:	Osteoprotegerin (TNFRSF11B Products)
Target Type:	Chemical
Background:	Osteoprotegerin (OGP) is a protein of 401 amino acids synthesized as a monomer of approximately 55 kDa. The mouse and human OGP proteins are approximately 85 percent and 94 percent identical with the rat protein, respectively. OPG belongs to the superfamily of TNF receptor-like proteins, having a strong similarity with the type-2 TNF receptor and CD40 and has been described as TNF receptor-like-1. OPGL appears to be an osteoclast differentiation and activation factor. The Human Osteoprotegerin ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Osteoprotegerin in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human Osteoprotegerin coated on a 96-well plate. Standards and samples are pipetted into the wells and Osteoprotegerin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human

Target Details

	Osteoprotegerin 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 Osteoprotegerin 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:	4982
UniProt:	O00300

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples2 - 10 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) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine. Suggested dilution for normal serum/plasma: 2-10 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. Add 200 µL Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 10 µL Osteoprotegerin standard from the vial of Item C, into a tube with 545.6 µL Assay Diluent A or

	1x Assay Diluent B to prepare a 900 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 200 µL 200 µL 200 µL 200 µL 200myl 10 µL standard + 545.6 µL 900 300 100 33.33 11.11 3.70 1.23 0 pg/mL 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 gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30

Application Details

	minutes at room temperature in the dark with gentle shaking. 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. Assay Diluent A Osteoprotegerin concentration (pg/mL) 0.1 1 10 100 1000 O D =4 50 n m 0.01 0.1 1 10 Assay Diluent B Osteoprotegerin concentration (pg/mL) 0.1 1 10 100 1000 O D =4 50 n m 0.01 0.1 1 10 Sensitivity: The minimum detectable dose of Osteoprotegerin is typically less than 1 pg/mL. Recovery: Recovery was determined by spiking various levels of human Osteoprotegerin into human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 99.85 89-108 Plasma 102.45 90-109 Cell culture media 99.75 89-108 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 97 98 97 Range (%) 91-108 88-109 88-108 1:4 Average % of Expected 96 95 95 Range (%) 92-108 88-109 91-108 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:	Bakry, El-Sayed, Hamza, Hassan: "Pretreatment levels of serum osteoprotegerin and p53 protein and urine telomerase as prognostic factors affecting survival in Egyptian bladder cancer

patients." in: **Oncology letters**, Vol. 11, Issue 1, pp. 823-830, (2016) (PubMed).

Kapelouzou, Tsourelis, Kaklamanis, Degiannis, Kogerakis, Cokkinos: "Serum and tissue biomarkers in aortic stenosis." in: **Global cardiology science & practice**, Vol. 2015, Issue 4, pp. 49, (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).

Lucas, Ramos, Prata, Rodrigues, Costa, Severo, Canhão, Fonseca, Barros: "Changes in serum RANKL and OPG with sexual development and their associations with bone turnover and bone mineral density in a cohort of girls." in: **Clinical biochemistry**, Vol. 47, Issue 12, pp. 1040-6, (2015) (PubMed).

Akelma, Cizmeci, Kanburoglu, Bozkaya, Catal, Mete, Kutukoglu, Namuslu: "Elevated level of serum osteopontin in school-age children with asthma." in: **Allergologia et immunopathologia**, Vol. 42, Issue 4, pp. 275-81, (2014) (PubMed).

There are more publications referencing this product on: Product page

Images

ELISA

Image 1.