Datasheet for ABIN625107

VEGFA ELISA Kit

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Overview

Quantity:	96 tests
Target:	VEGFA
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	10-6000 pg/mL
Minimum Detection Limit:	10 pg/mL
Application:	ELISA

Product Details

Purpose:	Human VEGF-A 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-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.
Sensitivity:	< 10 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments

Product Details

	• 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:	VEGFA
Alternative Name:	VEGF-A (VEGFA Products)
Background:	VEGF (Vascular endothelial growth factor) is also called VEGF-A, following the identification of several VEGF-related factors (VEGF-B, VEGF-C, VEGF-D, VEGF-E). VEGF significantly influence vascular permeability and is a strong angiogenic protein in several Bioassays and probably also plays a role in neovascularisation under physiological conditions. VEGF plays a role in the development and function of primate follicles and the ovarian corpus luteum, supporting the proliferation of blood vessels. The differentiation of adipocytes, of pheochromocytomas, and myocytes is accompanied by the controlled expression of VEGF. It has been demonstrated that inhibition of VEGF activity by treatment with a monoclonal antibody specific for VEGF can suppress tumor growth in vivo. The Human VEGF ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human VEGF in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human VEGF coated on a 96-well plate. Standards and samples are pipetted into the wells and VEGF present in a sample is bound to the wells by the immobilized

Target Details

	antibody. The wells are washed and biotinylated anti-human VEGF 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 VEGF 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:	7422
UniProt:	P15692
Pathways:	RTK Signaling, Glycosaminoglycan Metabolic Process, Regulation of Cell Size, Tube Formation, Signaling Events mediated by VEGFR1 and VEGFR2, Platelet-derived growth Factor Receptor Signaling, VEGFR1 Specific Signals, VEGF Signaling

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples2 - 5 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: 2-5 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 640 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 60 µL 50 ng/mL VEGF standard from the vial of Item C, into a tube with 440 µL Assay Diluent A or 1x Assay Diluent B to prepare a 6,000 pg/mL 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 200 µL 60 µL standard + 440 µL 6,000 2,000 666.7 222.2 74.07 24.69 8.23 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 100-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 300-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 40 µL of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a 300-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

Application Details

	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 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 Human VEGF concentration (pg/mL) O D =4 50 n m 0.001 0.01 0.1 1 10 0 10 100 1,000 10,000 Assay Diluent B Human VEGF concentration (pg/mL) O D =4 50 n m 0.001 0.01 0.1 1 10 0 10 100 1,000 10,000 Sensitivity: The minimum detectable dose of VEGF is typically less than 10 pg/mL. Recovery: Recovery was determined by spiking various levels of human VEGF into human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 104.4 92-115 Plasma 105.7 93-114 Cell culture media 103.5 92-113 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 96 97 97 Range (%) 92-113 91-114 90-111 1:4 Average % of Expected 97 96 102 Range (%) 91-112 88-108 91-113 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

Product cited in:

Etulain, Mena, Meiss, Frechtel, Gutt, Negrotto, Schattner: "An optimised protocol for platelet-rich plasma preparation to improve its angiogenic and regenerative properties." in: **Scientific reports**, Vol. 8, Issue 1, pp. 1513, (2018) (PubMed).

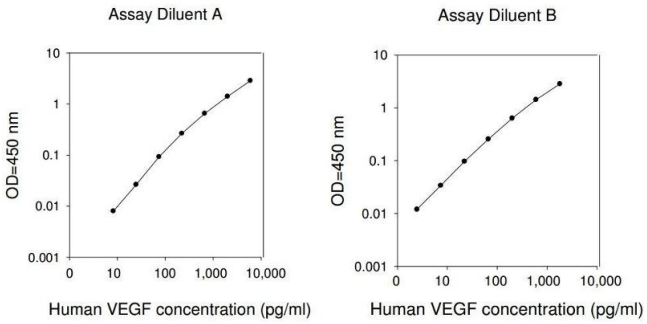
Yildirim, Dikmen, Terek, Akman, Gunel, Aktan, Zekioglu, Gunduz: "Do preoperative serum vascular endothelial growth factor and migration-inhibitory factor predict the nature of the adnexal masses? A prospective-controlled trial." in: **Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology**, Vol. 36, Issue 4, pp. 533-7, (2017) (PubMed).

Zeng, Whitmore, Sohn, Riker, Wiley, Scheetz, Stone, Tucker, Mullins: "Molecular response of chorioretinal endothelial cells to complement injury: implications for macular degeneration." in: **The Journal of pathology**, Vol. 238, Issue 3, pp. 446-56, (2016) (PubMed).

Joshi, Gupta, Khan, Kumar, Sharma, Kumar, Sharma: "Interrelationship between angiogenesis, inflammation and oxidative stress in Indian patients with multiple myeloma." in: **Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico**, Vol. 18, Issue 2, pp. 132-7, (2016) (PubMed).

Gamal, Abdel-Ghaffar, Iacono et al.: "Gingival crevicular fluid vascular endothelial cell growth factor and platelet-derived growth factor-BB release profile following the use of perforated barrier membranes during treatment of intrabony ..." in: **Journal of periodontal research**, Vol. 51, Issue 3, pp. 407-16, (2016) (PubMed).

There are more publications referencing this product on: Product page



ELISA

Image 1.