

Datasheet for ABIN625108

VEGFA ELISA Kit

1 Image

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Overview

Quantity:	96 tests
Target:	VEGFA
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Human VEGF-A ELISA Kit for cell and tissue lysate samples.
Sample Type:	Cell Lysate, Tissue Lysate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes human VEGF-A.
Sensitivity:	10 pg/mL
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

Product Details

- 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 • Cell lysate buffer
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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 cell lysate and tissue lysate. 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 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%.

Target Details

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

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: Tissue lysate and cell lysate sample should be diluted at least 5-fold with 1x Sample Diluent Buffer (Item D). 3. Sample Diluent Buffer (Item D) and Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C. Add 640 µl 1x Sample Diluent Buffer (Item D) 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 1x Sample Diluent Buffer to prepare a 6,000 pg/ml stock standard solution. Pipette 400 µl 1x Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Sample Diluent Buffer serves as the zero standard (0 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 into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be

Application Details

stored at 4°C for 5 days). The detection antibody concentrate should be diluted 100-fold with 1x Assay Diluent and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 15,000-fold with 1x Assay Diluent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 2 µl of HRP-Streptavidin concentrate into a tube with 198.0 µl 1x Assay Diluent to prepare a 100-fold diluted HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix through and then pipette 100 µl of prepared 100-fold diluted solution into a tube with 15 ml 1x Assay Diluent to prepare a final 15,000 fold diluted HRP-Streptavidin solution. 8. Cell lysate buffer should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

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 µl) 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 3. 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 3. 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.

Restrictions:

For Research Use only

Handling

Storage:

-20 °C

Storage Comment:

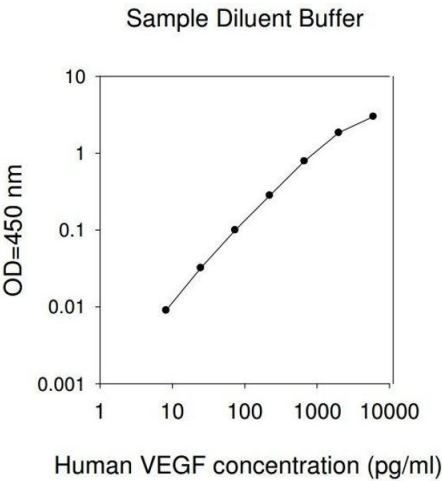
The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated

Handling

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

Images



ELISA

Image 1.