Datasheet for ABIN625219

VEGFA ELISA Kit

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Overview

Quantity:	96 tests
Target:	VEGFA
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	2-200 pg/mL
Minimum Detection Limit:	2 pg/mL
Application:	ELISA

Product Details

Purpose:	Rat 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 rat VEGF.
Sensitivity:	2 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

Product Details

	• 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 • Cell lysate buffer

Target Details

Target:	VEGFA
Alternative Name:	VEGF-A (VEGFA Products)
Background:	Vascular endothelial growth factor A (VEGF-A) (Vascular permeability factor) (VPF)
Gene ID:	83785
UniProt:	P16612
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. 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 400 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 4 µL VEGF standard from the vial of Item C, into a tube with 996.0 µL Sample Diluent Buffer to prepare a 200 pg/mL stock standard solution. Pipette 300 µL 1x Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. 1x Sample Diluent Buffer serves as the zero standard (0 pg/mL). 200 µL 200 µL 200 µL 200myl 200 µL 200 µL 4 µL standard + 996 µL 200 80 32 12.8 5.12 2.05 0.82 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 Diuent 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 Diuent 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 120-fold with 1x Assay Diuent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 100 µL of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent to prepare a 120 fold diluted HRP- Streptavidin solution. Mix well. 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. We recommend using 50-500 myg/mL of total protein for lysate sample. The amount of sample used depends on the abundance of target protein. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further. 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 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. Sample Diluent Buffer Rat VEGF 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 VEGF is typically less than 2 pg/mL. Recovery: Recovery was determined by spiking various levels of rat VEGF into rat tissue lysate and cell lysate. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Tissue lysate 94.34 82-102 Cell lysate 92.67 81-102 Linearity: Sample Type Tissue Cell Lysate lysate 1:2 Average % of 92 93 Expected Range (%) 82-102 83-104 1:4 Average % of 94 95 Expected Range (%) 83-103 84-103 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:

Yüksel, Yavuz, I?, Çomuno?lu, Üzüm, Akyüz, Y?ld?r?m: "Simvastatin reduces VEGF and NO levels in acute stages of experimental traumatic brain injury." in: **Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology**, Vol. 34, Issue 11, pp. 1941-6, (2013) (PubMed).

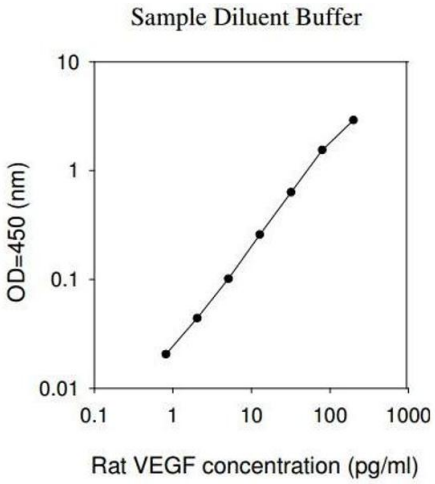
Dharmani, De Simone, Chadee: "The probiotic mixture VSL#3 accelerates gastric ulcer healing by stimulating vascular endothelial growth factor." in: **PLoS ONE**, Vol. 8, Issue 3, pp. e58671, (2013) (PubMed).

De Laporte, des Rieux, Tuinstra, Zelivyanskaya, De Clerck, Postnov, Préat, Shea: "Vascular endothelial growth factor and fibroblast growth factor 2 delivery from spinal cord bridges to enhance angiogenesis following injury." in: **Journal of biomedical materials research. Part A**, Vol. 98, Issue 3, pp. 372-82, (2011) (PubMed).

Tolstanova, Deng, Khomenko, Garg, Paunovic, Chen, Sitaraman, Shiloach, Szabo, Sandor: "Role of anti-angiogenic factor endostatin in the pathogenesis of experimental ulcerative colitis." in: **Life sciences**, Vol. 88, Issue 1-2, pp. 74-81, (2010) (PubMed).

Cohen, Gitay-Goren, Neufeld, Levi: "High levels of biologically active vascular endothelial growth factor (VEGF) are produced by the baculovirus expression system." in: **Growth factors (Chur, Switzerland)**, Vol. 7, Issue 2, pp. 131-8, (1992) (PubMed).

There are more publications referencing this product on: Product page



ELISA

Image 1.