Datasheet for ABIN1379960

VEGF ELISA Kit

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Overview

Quantity:	96 tests
Target:	VEGF
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	32-2000 pg/mL
Minimum Detection Limit:	32 pg/mL
Application:	ELISA

Product Details

Purpose:	The OmniKine? Rat VEGF ELISA Kit contains the components necessary for quantitative determination of natural or recombinant Rat VEGF concentrations within any experimental sample including cell lysates, serum and plasma. This particular immunoassay utilizes the quantitative technique of a "Sandwich" Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a "sandwich" format by the primary capture antibodies coated to each well-bottom and the secondary detection antibodies added subsequently by the investigator. The capture antibodies coated to the bottom of each well are specific for a particular epitope on Rat VEGF while the user-added detection antibodies bind to epitopes on the captured target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non- specific binding between proteins to other proteins or to the solid phase. After incubation and "sandwiching" of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetric reaction to ensue upon substrate addition. When the substrate TMB (3, 3', 5, 5'-Tetramethylbenzidine) is added, the

	reaction catalyzed by peroxidase yields a blue color that is representative of the antigen concentration. Upon sufficient color development, the reaction can be terminated through addition of Stop Solution (2 N Sulfuric Acid) where the color of the solution will turn yellow. The absorbance of each well can then be read by a spectrophotometer, allowing for generation of a standard curve and subsequent determination of protein concentration.
Brand:	OmniKine™
Sample Type:	Cell Lysate, Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The Rat VEGF ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Rat VEGF proteins.
Cross-Reactivity (Details):	The Rat VEGF ELISA is capable of recognizing both recombinant and naturally produced Rat VEGF proteins. The antigens listed below were tested at 50 ng/mL and exhibited 100% cross reactivity. Human: VEGF121, VEGF165 Murine: VEGF The antigens listed below were tested at 50 ng/mL and did not exhibit significant cross reactivity or interference. Human: VEGF-B
Characteristics:	The Rat VEGF ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Rat VEGF proteins within the range of 32-2000 pg/mL.
Components:	• Microstrips Coated w / Capture Antibody: 12 x 8-Well Microstrips • Protein Standard: Lyophilized (100 ng), Red container • Biotinylated Detection Antibody: Lyophilized, Yellow container • 400x Streptavidin-HRP: 30 µL, Blue container • Wash Buffer (10x): 50 mL, Clear containter • Assay Diluent: 50 mL, Clear container • Ready-to-Use Substrate: 12 mL, Brown container • Stop Solution: 12 mL, Clear container • Adhesive Plate Sealers: 4 Sheets • Technical Manual 1 Manual
Material not included:	The following materials and/or equipment are NOT provided in this kit but are necessary to successfully conduct the experiment: Microplate reader able to measure absorbance at 450 nm (with correction wavelength set to 540 nm or 570 nm) Micropipettes with capability of measuring volumes ranging from 1 µl to 1 mL Deionized or sterile water Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate

Product Details

	washer
	Graph paper or computer software capable of generating or displaying logarithmic functions
	Absorbent paper or vacuum aspirator
	Test tubes or microfuge tubes capable of storing ≥1 mL
	Bench
	top centrifuge (optional)
	Bench
	top vortex (optional)
	Orbital shaker (optional)

Target Details

Target:	VEGF
Alternative Name:	VEGF (VEGF Products)
Background:	Rat VEGF or Vascular Endothelial Growth Factor, also known as Vascular Permeability Factor, is a 214 amino acid cytokine protein encoded by the Vegfa gene located at locus 9q12 on chromosome 9. After initial synthesis and translocation, the 26 residue signal sequence is cleaved from the N-terminal end, allowing for proper folding and maturation of the VEGF peptide. VEGF, a homodimeric member of the PDGF/VEGF growth factor family, is a growth factor active in angiogenesis, vasculogenesis and endothelial cell growth. It also has a major role in inducing endothelial cell proliferation, promoting cell migration, inhibiting apoptosis and inducing permeabilization of blood vessels. Through binding to the FLT1/VEGFR1 and KDR/VEGFR2 receptors, heparin sulfate and heparin, VEGF may play a role in increasing vascular permeability during lactation, when increased transport of molecules from the blood is required for efficient milk protein synthesis. There are several isoforms of VEGF that are produced. VEGF- A120 is acidic and freely secreted. VEGF-A164 is more basic, has heparin-binding properties and, although a significant proportion remains cell- associated, most is freely secreted. VEGF-A188 is very basic, it is cell- associated after secretion and is bound avidly by heparin and the extracellular matrix, although it may be released as a soluble form by heparin, heparinase or plasmin. The cytokine is expressed in the pituitary, in brain, in particularly in supraoptic and paraventricular nuclei and the choroid plexus. VEGF is also found abundantly in the corpus luteum of the ovary, in kidney glomeruli, in the ductal epithelial cells of post-pubertal mammary glands, and in ductal and alveolar epithelial cells. Source: Entrez Gene: Vegfa vascular endothelial growth factor A [Rattus norvegicus]: Swiss-Prot: P16612
UniProt:	P16612

Application Details

Plate:	Pre-coated
Protocol:	This particular immunoassay utilizes the quantitative technique of a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a sandwich format by the primary capture antibodies coated to each well-bottom and the secondary detection antibodies added subsequently by the investigator. The capture antibodies coated to the bottom of each well are specific for a particular epitope on the Rat VEGF cytokine while the user-added detection antibodies bind to epitopes on the captured target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non-specific binding between proteins to other proteins or to the solid phase. After incubation and sandwiching of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetric reaction to ensue upon substrate addition. When the substrate TMB (3, 3', 5, 5'- Tetramethylbenzidine) is added, the reaction catalyzed by peroxidase yields a blue color that is representative of the antigen concentration. Upon sufficient color development, the reaction can be terminated through addition of Stop Solution (2 N Sulfuric Acid) where the color of the solution will turn yellow. The absorbance of each well can then be read by a spectrophotometer, allowing for generation of a standard curve and subsequent determination of protein concentration.
Sample Preparation:	If samples are to be used within 24 hours, aliquot and store at 4 °C. If samples are to be used over a long period of time, aliquot and store between -20 °C and -80 °C, depending on the duration of storage. Note: Samples containing a visible precipitate or pellet must be clarified prior to use in the assay. Caution: Avoid repeated freeze/thaw cycles to prevent loss of biological activity of proteins in experimental samples. • Cell Lysate and Supernatants: Remove large cell components via centrifugation and perform the assay. Cell lysates and supernatants require a dilution using Assay Diluent. A serial dilution may be performed to determine a suitable dilution factor for the sample. For future use of the sample, follow the sample storage guidelines stated above. • Serum: Allow samples to clot in a serum separator tube (SST) for 30 minutes. After sufficient clotting, centrifuge at 1000 x g for 15 minutes and remove serum from SST in preparation for the assay. Serum samples require at least a 1:50 dilution using Assay Diluent. For future use of the sample, follow the storage guidelines above. • Plasma: Use heparin, citrate or EDTA as an anticoagulant to gather plasma from original biological

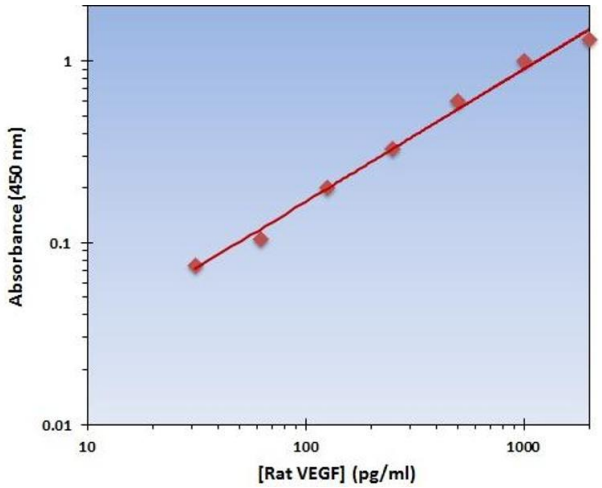
Application Details

	sample. After collection of the plasma, centrifuge for 15 minutes at 1000 x g. This step must be performed within 30 minutes of plasma collection. Plasma samples require at least a 1:50 dilution using Assay Diluent. Afterwards, perform the assay or for future use of the sample, follow the storage guidelines stated above.
Assay Procedure:	Note: If possible, all incubation steps should be performed on an orbital shaker to equilibrate solutions when added to the microplate wells. Also, all provided solutions should be at ambient temperature prior to use. Note: Avoid adding solutions into wells at an angle, always keep pipette tip perpendicular to plate bottom. Reconstitution of Provided Materials: 1. Reconstitute the Biotin-Conjugated Detection Antibody in 67 µL of ddH□O for a concentration of 180 µg/ml. 2. Reconstitute the Protein Standard in 100 µL of ddH□O for a concentration of 340 ng/ml. 3. Dilute the 50 mL of 10x Wash Buffer in 450 mL of ddH2O for 500 mL of 1x Wash Buffer. Addition of Known Standard and Unknown Sample to Immunoassay: The OmniKine™ Human CD163 ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human CD163 proteins
Calculation of Results:	Generation of Standard Curve and Interpretation of Data 1. Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard optical density. 2. Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4- Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis). Note: Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or trend-line through the plotted points via regression analysis.
Restrictions:	For Research Use only

Handling

Precaution of Use:	Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment. Stop Solution contains 2 N Sulfuric Acid (H2SO4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is

	finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.
Handling Advice:	This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind. Materials included in this kit should NOT be used past the expiration date on the kit label. Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits. Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided. The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit. Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment. Stop Solution contains 2 N Sulfuric Acid (H2SO4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.
Storage:	4 °C
Storage Comment:	Note: If used frequently, reagents may be stored at 4 °C. • Unopened Kits: Store at 4 °C for 6 months. • Microstrips Coated w/ Capture Antibody, 400x Streptavidin-HRP Wash Buffer (10x), Assay Diluent Ready-to-Use Substrate, Stop Solution: 6 Months at 4 °C • Protein Standard, Biotinylated Detection Antibody: Lyophilized: 6 Months (if Reconstituted: 1 Month) at 4 °C



ELISA

Image 1. This is an example of what a typical standard curve will look like. You must make your own standard curve. Do not use this example as your own standard curve.