

Datasheet for ABIN625187

VEGF ELISA Kit[Go to Product page](#)**1** Image**6** Publications

Overview

Quantity: 96 tests

Target: VEGF

Reactivity: Mouse

Method Type: Sandwich ELISA

Application: ELISA

Product Details

Purpose: Mouse 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 mouse VEGF.

Cross-Reactivity (Details): This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., Mouse 6Ckine, CTACK, Eotaxin, GCSF, GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17, IFN-gamma, KC, Leptin, MCP-5, MIP-1alpha, MIP-2, MIP-3beta, RANTES, SCF, sTNF α , TARC, TIMP-1, TNF-alpha, Tpo).

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

Product Details

Components:	<ul style="list-style-type: none">• 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
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Material not included:	<ul style="list-style-type: none">• 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:	VEGF
Alternative Name:	VEGF (VEGF Products)
Background:	Vascular endothelial growth factor A (VEGF-A) (Vascular permeability factor) (VPF)
Gene ID:	22339
UniProt:	Q00731

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	<ol style="list-style-type: none">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 25 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 40 µL VEGF standard from the vial of Item C, into a tube with 960.0 µL Sample Diluent Buffer to prepare a 1,000 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	200 µL	200 µL	200 µL	40 µL standard	+ 960 µL
1,000	400	160	64	25.6	10.2	4.1	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 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 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 160-fold with 1x Assay Diluent. 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 16 ml 1x Assay Diluent to prepare a 160-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). 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 µg/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 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
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 Mouse VEGF concentration (pg/mL) 1 10 100 1000 10000 O D = 4 50 n m 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 mouse VEGF into mouse tissue lysate and cell lysate. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Tissue lysate 87.87 81-102 Cell lysate 88.62 82-103

Linearity: Sample Type Tissue Cell Lysate lysate 1:2 Average % of 92 93 Expected Range (%) 82-102 83-103 1:4 Average % of 95 96 Expected Range (%) 84-103 85-105

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.

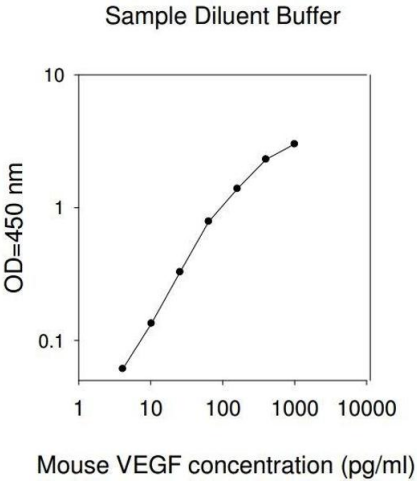
Handling

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 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:	<p>Martin, Nguyen, Molinolo, Gutkind: "Accumulation of dephosphorylated 4EBP after mTOR inhibition with rapamycin is sufficient to disrupt paracrine transformation by the KSHV vGPCR oncogene." in: Oncogene, Vol. 33, Issue 18, pp. 2405-12, (2014) (PubMed).</p> <p>Said, Frierson, Sanchez-Carbayo, Brekken, Theodorescu: "Loss of SPARC in bladder cancer enhances carcinogenesis and progression." in: The Journal of clinical investigation, Vol. 123, Issue 2, pp. 751-66, (2013) (PubMed).</p> <p>Degoul, Borel, Jacquemot, Besse, Communal, Mishellany, Papon, Penault-Llorca, Donnarieix, Doly, Maigne, Miot-Noirault, Cayre, Cluzel, Moins, Chezal, Bonnet: "In vivo efficacy of melanoma internal radionuclide therapy with a ¹³¹I-labelled melanin-targeting heteroarylcarboxamide molecule." in: International journal of cancer. Journal international du cancer, Vol. 133, Issue 5, pp. 1042-53, (2013) (PubMed).</p> <p>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).</p> <p>Ferrara, Houck, Jakeman, Winer, Leung: "The vascular endothelial growth factor family of polypeptides." in: Journal of cellular biochemistry, Vol. 47, Issue 3, pp. 211-8, (1992) (PubMed).</p>
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There are more publications referencing this product on: [Product page](#)



ELISA

Image 1.