Version 5d Last updated 20 September 2021

ab100662
VEGF Human ELISA Kit

For the quantitative measurement of human VEGF in serum, plasma and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Abcam's VEGF Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Human VEGF in serum, plasma and cell culture supernatants.

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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standard or sample to appropriate wells.

Incubate at room temperature.

Wash and add prepared biotin antibody to each well. Incubate at room temperature.

Wash and add prepared Streptavidin Solution. Incubate at room temperature.

Add TMB One-Step Development Solution to each well. Incubate at room temperature

Add Stop Solution to each well. Read at 450 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
VEGF Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer	25 ml	-20°C
Assay Diluent A	30 ml	-20°C
5X Assay Diluent B	15 ml	-20°C
Biotinylated anti-Human VEGF	2 vials	-20°C
Recombinant Human VEGF Standard	2 vials	-20°C
300X HRP-Streptavidin Concentrate	200 µl	-20°C
TMB One-Step Substrate Reagent	12 ml	-20°C
Stop Solution	8 ml	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Ensure plates are properly sealed or covered during incubation steps.
- When preparing your standards, it is critical to briefly centrifuge the vial first. The powder may adhere to the cap and not be included in the standard solution resulting in an incorrect concentration. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and centrifuge briefly; repeat this procedure 3-4 times. This is an effective technique for thorough mixing of the standard without using excessive mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.

- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- Make sure the microplate reader is switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Assay Diluent B

5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before.

9.2 1X Wash Solution

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 ml of 20X Wash Solution Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Solution.

9.3 1X Biotinylated VEGF Detection Antibody

Briefly spin the Biotinylated anti-Human VEGF vial 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 either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate must be diluted 100-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly spin the 300X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. HRP Streptavidin concentrate must be diluted 300-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 40 µL of 300X HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent B to prepare a final 300 fold diluted 1X HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. Standard Preparation

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Standard (recombinant protein) should be stored at -80°C after reconstitution for up to 7 days.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Briefly spin the vial of VEGF Standard. Prepare the 50 ng/mL **Stock Standard** by adding 640 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium) into the vial (see table below).

10.2 Ensure the powder is thoroughly dissolved by gentle mixing.

10.3 Label tubes #1 – 8.

10.4 Prepare **Standard #1** by adding 60 µL of the 50 ng/mL **Stock Standard**, to 440 µL of Assay Diluent A or 1X Assay Diluent B into tube 1#. Mix thoroughly and gently.

10.5 Pipette 400 µL Assay Diluent A or 1X Assay Diluent B into each tube.

10.6 Prepare **Standard #2** by adding 200 µL Standard #1 to tube #2 and mix thoroughly.

10.7 Prepare **Standard #3** by adding 200 µL Standard #2 to tube #3 and mix thoroughly.

10.8 Using the table below as a guide, prepare subsequent serial dilutions. Standard #8 contains no protein and is the Blank control.

Standard #	Volume to dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	60	440	500	50000	6000
2	200	400	600	6000	2000
3	200	400	600	2000	666.7
4	200	400	600	666.7	222.2
5	200	400	600	222.2	74.1
6	200	400	600	74.1	24.69
7	200	400	600	24.69	8.23
8 (Blank)	0	400	400	0	0

11. Sample Preparation

- If your samples need to be diluted, Assay Diluent A should be used for dilution of serum/plasma samples. 1X Assay Diluent B should be used for dilution of culture supernatants.
- 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.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Add 100 µl of each standard (see Standard Preparation section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

13.2 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (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 1X Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

13.3 Add 100 µL of 1X Biotinylated VEGF Detection Antibody (Reagent Preparation, section 9.3) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of 1X HRP-Streptavidin solution (see Reagent Preparation section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

13.8 Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

13.9 Analyze the data as described below.

13.9.1 Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average Blank absorbance value.

13.9.2 Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

13.9.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

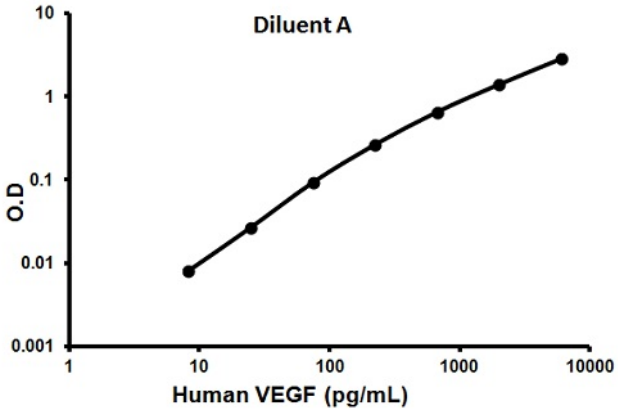

Figure 1. Example of human VEGF standard curve in Diluent A. The standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

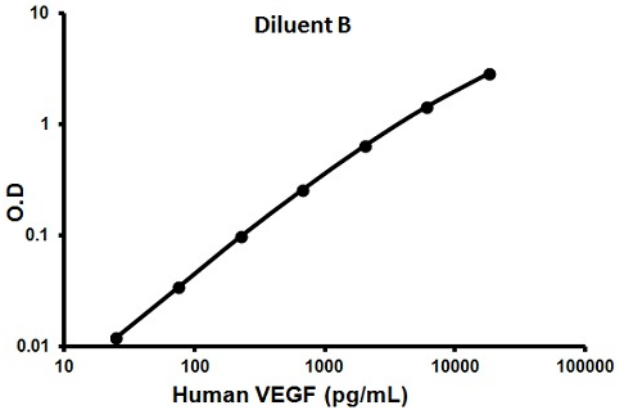

Figure 2. Example of human VEGF standard curve in Diluent B. The standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Conc. (pg/mL)	O.D.	
	Assay Diluent A	Assay Diluent B
8.23	0.008	0.012
24.69	0.027	0.034
74.07	0.093	0.097
222.2	0.265	0.257
666.7	0.653	0.636
2,000	1.407	1.431
6,000	2.858	2.848

15. Typical Sample Values

SENSITIVITY –

The minimum detectable dose (MDD) of VEGF is typically less than 10 pg/mL.

PRECISION –

	Intra-Assay	Inter-Assay
CV (%)	<10%	<12%

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 of Dilution

Serum Dilution	Average % Expected Value	Range (%)
1:2	96	92-113
1:4	97	91-112

Plasma Dilution	Average % Expected Value	Range (%)
1:2	97	91-114
1:4	96	88-108

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	97	90-111
1:4	102	91-113

16. Assay Specificity

The antibodies used within this ELISA kit detect human VEGF.

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., Human Angiogenin, BDNF, BLC, ENA-78, FGF-4,IL-1α, IL-1β, 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, IL-309, IP-10, G-CSF, GM-CSF, IFN-γ, Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1α, MIP-1β, MIP-1δ, PARC,PDGF, RANTES, SCF, TARC, TGF-β, TIMP-1, TIMP-2, TNF- α,TNF-β,TPO).

17. Species Reactivity

This kit recognizes human VEGF.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)