

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSTICS !)

Catalog No: E-TSEL-H0026

Product size: 96T/48T/24T/96T*5

QuicKey Human VEGF-A(Vascular Endothelial Growth Factor A) ELISA Kit

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help (info in the header of each page).

Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label on the side of box.

Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

QuicKey Series

Get more sensitive and precise results with saving at least 1h comparing to traditional ELISA Kits. The new developed technology in house will help to accelerate your science research in a more efficient way.

Intended use

This ELISA kit applies to the in vitro quantitative determination of Human VEGF-A concentrations in serum, plasma. Please consult technical support for the applicability if other biological fluids need to be tested.

Specification

- Sensitivity: 0.03ng/mL.
- Detection Range: 0.04-10ng/mL
- Specificity: This kit recognizes Human VEGF-A in samples. No significant cross-reactivity or interference between Human VEGF-A and analogues was observed.
- Repeatability: Coefficient of variation is < 10%.

Background

Vascular endothelial growth factor A(VEGF-A) is a multifunctional cytokine that exerts in vivo a key role in physiological and pathological neoangiogenesis by stimulating endothelial cell proliferation and vessel hyperpermeability.[1] It is a member of the platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF) family and encodes a protein that is often found as a disulfide linked homodimer. In human, VEGF-A is produced by a group of isoforms as a result of alternative splicing and VEGF189, VEGF165, VEGF121 are widely expressed. Elevated levels of this protein is linked to POEMS syndrome, mutations in this gene have been associated with proliferative and nonproliferative diabetic retinopathy.[2] VEGF-A also has a major role in the pathogenesis of many other diseases including hypervascularized tumors, rheumatoid arthritis, cutaneous diseases. [3]

1. Murphy J F , Fitzgerald D J . Vascular endothelial growth factor induces cyclooxygenase-dependent proliferation of endothelial cells via the VEGF-2 receptor[J]. The FASEB Journal, 2001, 15(9):1667-1669.
2. Watanabe D, Suzuma K, Suzuma I, et al. Vitreous levels of angiopoietin 2 and vascular endothelial growth factor in patients with proliferative diabetic retinopathy[J]. American journal of ophthalmology, 2005, 139(3): 476-481.
3. Dixelius J , Olsson A K , Thulin A , et al. Minimal Active Domain and Mechanism of Action of the Angiogenesis Inhibitor Histidine-Rich Glycoprotein[J]. Cancer Research, 2006, 66(4):2089-2097.

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human VEGF-A. Samples (or Standards) and biotinylated detection antibody specific for Human VEGF-A are added to the micro ELISA plate wells. Human VEGF-A would combine with the specific antibody. Then Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human VEGF-A, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of Human VEGF-A. You can calculate the concentration of Human VEGF-A in the samples by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 2-8°C for six months. After test, the unused wells and reagents should be stored according to the table below.

| Item | Specifications | Storage |
|--|---|--|
| Micro ELISA Plate (Dismountable) | 96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips 96T*5: 5 plates, 96T | 2-8°C, 1 month |
| Reference Standard | 96T: 2 vials 48T/24T: 1 vial 96T*5: 10 vials | 2-8°C, use the reconstituted standard within 24h |
| Reference Standard & Sample Diluent | 96T/48T/24T: 1 vial, 20 mL 96T*5: 5 vials, 20 mL | 2-8°C |
| Biotinylated Detection Ab Working Solution | 96T/48T/24T: 1 vial, 6 mL 96T*5: 5 vials, 6 mL | |
| HRP Conjugate Diluent | 96T/48T/24T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL | |
| Concentrated Wash Buffer(25×) | 96T/48T/24T: 1 vial, 30 mL 96T*5: 5 vials, 30 mL | |
| Concentrated HRP Conjugate (100×) | 96T: 1 vial, 120 µL 48T/24T: 1 vial, 60 µL 96T*5: 5 vials, 120 µL | 2-8°C(Protect from light) |
| Substrate Reagent | 96T/48T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL | |
| Stop Solution | 96T/48T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL | 2-8°C |
| Plate Sealer | 96T/48T/24T: 5 pieces 96T*5: 25 pieces | |
| Product Description | 1 copy | |
| Certificate of Analysis | 1 copy | |

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

Microplate reader with 450nm wavelength filter

High-precision transfer pipette, EP tubes and disposable pipette tips

Incubator capable of maintaining 37°C

Deionized or distilled water

Absorbent paper

Loading slot for Wash Buffer

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA Plate may appear to have a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch provided in the kit, store it according to the conditions suggested in the above table.
3. Do not reuse the reconstituted standard and HRP conjugate working solution. The unspent biotinylated detection Ab working solution and other stock solutions should be stored according to the storage conditions suggested in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450 ± 2 nm. The optical density should be within 0~3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
5. Do not mix or use components with those from other lots.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

(More detailed information please view our website: <http://www.elabscience.com/List-detail-253.html>)

Serum: Allow samples to clot for 1 hour at room temperature or overnight at 2-8°C before centrifugation for 15 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

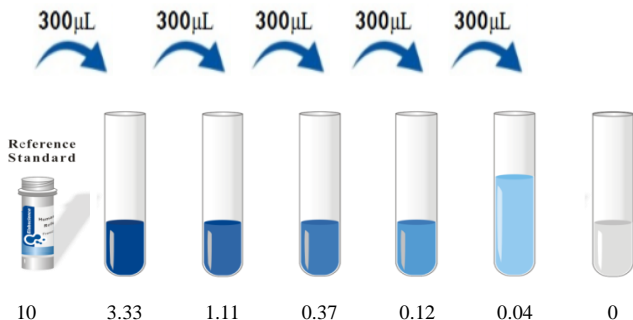
Note for sample

1. Tubes for blood collection should be disposable and be non-endotoxin. Samples with high hemolysis or much lipid are not suitable for ELISA assay.
2. Samples should be assayed within 7 days when stored at 2-8°C, otherwise samples must be divided up and stored at -20°C (≤ 1 month) or -80°C (≤ 3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates. Bring samples to room temperature and mix gently.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
4. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
5. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.
6. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25°C) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** Dilute 30mL of Concentrated Wash Buffer with 720mL of deionized or distilled water to prepare 750mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 10ng/mL (or add 1.0mL of Reference Standard & Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 10、3.33、1.11、0.37、0.12、0.04、0ng/mL.

Dilution method: Take 7 EP tubes, add 600 μ L of Reference Standard & Sample Diluent to each tube. Pipette 300 μ L of the 10ng/mL working solution to the first tube and mix up to produce a 3.33ng/mL working solution. Pipette 300 μ L of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.



4. **HRP Conjugate working solution:** HRP Conjugate is HRP conjugated avidin. Calculate the required amount before the experiment (100 μ L/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800 \times g for 1 min, then dilute the 100 \times Concentrated HRP Conjugate to 1 \times working solution with HRP Conjugate Diluent..

Assay procedure (A brief assay procedure is on the 12th page)

- Determine wells for **diluted standard**, **blank** and **sample**. Add 50 μ L each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Immediately add 50 μ L of **Biotinylated Detection Ab working solution** to each well. Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37 $^{\circ}$ C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
- Aspirate or decant the solution from each well, add 350 μ L of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
- Add 100 μ L of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37 $^{\circ}$ C.

- Aspirate or decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.
- Add 90 μ L of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37 °C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min. Preheat the Microplate Reader for about 15 min before OD measurement.
- Add 50 μ L of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
- Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation of results

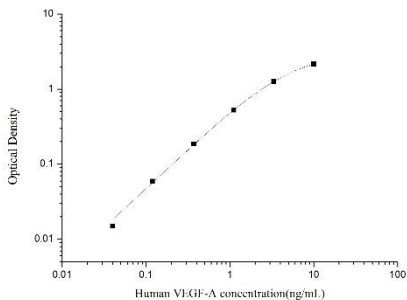
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

| | | | | | | | |
|----------------------|-------|-------|-------|-------|-------|-------|-------|
| Concentration(ng/mL) | 10 | 3.33 | 1.11 | 0.37 | 0.12 | 0.04 | 0 |
| OD | 2.244 | 1.337 | 0.602 | 0.262 | 0.136 | 0.092 | 0.077 |
| Corrected OD | 2.167 | 1.26 | 0.525 | 0.185 | 0.059 | 0.015 | - |



Sample values

Serum/Plasma—Samples from apparently healthy volunteers were evaluated for the presence of Human VEGF-A in this assay.

| Sample Type | Source | Range | Dilution Factor |
|--------------------|---------------|------------------|-----------------|
| Serum (n=12) | Healthy human | 52.6-341.18pg/mL | 1-8 |
| EDTA plasma (n=12) | Healthy human | ND-282.01pg/mL | 1-8 |

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Human VEGF-A were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Human VEGF-A were tested on 3 different plates, 20 replicates in each plate.

| Sample | Intra-assay Precision | | | Inter-assay Precision | | |
|--------------------|-----------------------|------|------|-----------------------|------|------|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 20 | 20 | 20 | 20 | 20 | 20 |
| Mean(ng/mL) | 0.48 | 1.13 | 4.28 | 0.42 | 1.19 | 4.51 |
| Standard deviation | 0.03 | 0.05 | 0.21 | 0.02 | 0.06 | 0.22 |
| CV (%) | 6.25 | 4.42 | 4.91 | 4.76 | 5.04 | 4.88 |

Recovery

The recovery of Human VEGF-A spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

| Sample Type | Range (%) | Average Recovery (%) |
|-------------------|-----------|----------------------|
| Serum (n=8) | 86-97 | 91 |
| EDTA plasma (n=8) | 82-96 | 90 |

Linearity

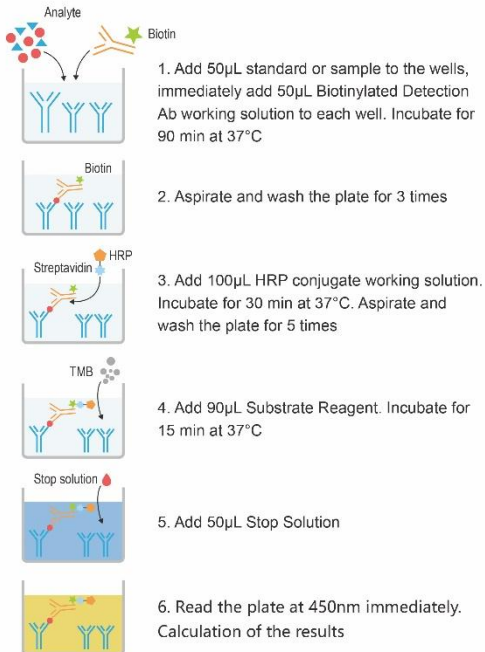
Samples were spiked with high concentrations of Human VEGF-A and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

| | | Serum (n=4) | EDTA plasma (n=4) |
|------|-------------|-------------|-------------------|
| 1:2 | Range (%) | 97-110 | 98-104 |
| | Average (%) | 103 | 101 |
| 1:4 | Range (%) | 97-110 | 84-94 |
| | Average (%) | 104 | 90 |
| 1:8 | Range (%) | 88-101 | 97-108 |
| | Average (%) | 93 | 105 |
| 1:16 | Range (%) | 86-95 | 101-108 |
| | Average (%) | 92 | 104 |

Troubleshooting

| Problem | Causes | Solutions |
|---------------------------------------|---|--|
| Poor standard curve | Inaccurate pipetting | Check pipettes. |
| | Improper standard dilution | Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing. |
| | Wells are not completely aspirated | Completely aspirate wells in between steps. |
| Low signal | Insufficient incubation time | Ensure sufficient incubation time. |
| | Incorrect assay temperature | Use recommended incubation temperature. Bring substrate to room temperature before use. |
| | Inadequate reagent volumes | Check pipettes and ensure correct preparation. |
| | Improper dilution | |
| HRP conjugate inactive or TMB failure | Mix HRP conjugate and TMB, rapid coloring. | |
| Deep color but low value | Plate reader setting is not optimal | Verify the wavelength and filter setting on the Microplate reader. |
| Large CV | Inaccurate pipetting | Check pipettes |
| High background | Concentration of target protein is too high | Use recommended dilution factor. |
| | Plate is insufficiently washed | Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. |
| | Contaminated wash buffer | Prepare fresh wash buffer. |
| Low sensitivity | Improper storage of the ELISA kit | All the reagents should be stored according to the instructions. |
| | Stop solution is not added | Stop solution should be added to each well before measurement. |

SUMMARY



Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven't compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.