Human Vascular Endothelial Cell Growth Factor Receptor 3 ELISA KIT

BIOENZY
Rev.04-1

Catalog no	**BZ-02011722-EB**

User Instruction

Standard Curve Range	0.16 ng/mL – 10 ng/mL
Sensitivity	0.1 ng/mL
Repeatability	Coefficient of variation is ≤ 10%
Storage	Unopened ELISA Kits can be stored at 2–8°C for 12 months. Avoid repeated freeze–thaw cycles. Do not use the kit beyond its expiration date.

Specificity

This kit is designed for the detection of Human VEGFR3/Flt4. It has been tested and shows no significant cross-reactivity with other similar substances. However, due to technical limitations and the variability of sample sources, it is not possible to test all related or similar substances for cross-reactivity. Therefore, this kit may potentially exhibit cross-reactivity with other substances that have not been tested.

Assay Principle

This ELISA kit uses the Sandwich-ELISA principle. The microplate provided in this kit has been pre-coated with an antibody specific to Human VEGFR3/Flt4. Samples (or Standards) are added to the microplate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human VEGFR3/Flt4 and 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 VEGFR3/Flt4, 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 nm. The OD value is proportional to the concentration of Human VEGFR3/Flt4. Calculate the concentration of Human VEGFR3/Flt4 in the samples by comparing the OD of the samples to the standard curve.

Storage Directions and Kit Components

Keep the **whole kit** before use in 2–8°C for 12 months.

After opening handling, store the items separately according to the following directions:

Components	96 Wells	48 Wells	Storage Directions After Opening Handling
Pre-Coated Microplate	12 x 8 wells	6 x 8 wells	-20°C (12 months)
Standard Solution	2 vials	1 vial	
Biotinylated Antibody (100x)	120 ul	60 ul	
HRP Conjugate (100x)	120 ul	60 ul	
Standard & Sample Diluent	20 ml	20 ml	2-8°C (12 months)
Biotinylated Antibody Diluent	13 ml	13 ml	
HRP Conjugate Diluent	13 ml	13 ml	
Wash Buffer (25x)	30 ml	30 ml	
Stop Solution	10 ml	10 ml	
Substrate Reagent	10 ml	10 ml	2-8°C (light sensitive, avoid light)
Plate Covers	5	5	RT
Instruction manual	1	1	

Notes:

- If the whole kit is stored at -20°C, place the kit at 2-8°C the day before the experiment.
- After receiving the kit, the plates can be disassembled to single strips. Please use it in batches on demands.

Material Required But Not Supplied

- 37°C incubator
- Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 nm wavelength filter

Precautions

- For your safety, always wear lab coats, goggles, and latex gloves. Conduct the experiment in line with national biosafety protocols for biological labs, particularly when handling blood samples or other bodily fluids.
- A newly opened ELISA plate might show a watery liquid, which is expected and won't affect results. Seal unused wells back in the foil pouch and store them as indicated in the table above.
- Never reuse the reconstituted standard, biotinylated detection antibody working solution, or concentrated HRP conjugate working solution. Store any remaining undiluted concentrated biotinylated detection antibody (100×) and other stock solutions per the table's conditions.
- Avoid using expired reagents. For multi-session use, prepare only the needed amounts of each component to minimize waste and support accurate future runs. It's best to retain the microplate frame, reseal unused wells in the aluminum foil bag, and use all items before their expiration dates.
- Do not interchange or mix reagents from different kits, batches, or suppliers with those in this kit.

- Do not reuse diluted standards, biotinylated antibody working solutions, or enzyme conjugate working solutions. The highest-concentration standard can be kept at -20°C for up to two weeks, but avoid repeated freeze-thaw cycles.
- Run all samples and standards in duplicate for best accuracy.

Specimen Collection

Serum Allow serum to clot for 120 minutes at room temperature or overnight at 2-8°C. Centrifuge at 2000xg for 15 minutes. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Plasma Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 minutes at 2000xg at 2-8°C within 30 minutes of collection. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Cell Culture Supernatant and other biological fluids Centrifuge samples for 15 min at 1500× g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Tissue homogenates Tissues should be rinsed in ice-cold PBS to remove excess blood thoroughly, weighed, minced into small pieces. then Tissue pieces homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. The resulting suspension is sonicated with an ultrasonic cell disrupter till the solution is clarified. The homogenates are then centrifuged for 5 min at 10000 x g. Supernatants should be taken for assay testing immediately or stored at -20°C or -80°C for later use.

Cell lysates For adherent cells, gently wash the cells with moderate amount of cool PBS and detach the cells with trypsin. Collect cells by centrifugation for 5 min at 1500×g (suspension cells can be collected by centrifugation directly). Discard the supernatant and wash cells 3 times with cool PBS. Resuspend cells in cool PBS with concentration of 5×106 cells/mL. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 15 min at 2000 x g at 2-8 °C. Supernatants should be taken for assay testing immediately or stored at -20°C or -80 °C for later use.

Note

- Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must **contact us** to

determine the optimal sample for their particular experiments.

- Blood collection tubes must be single-use, free of pyrogens, and endotoxin-free. Avoid freezing whole blood samples directly; instead, process them into serum or plasma before testing or storage.
- Samples to be used within 7 days should be stored at 2-8℃. Samples should be aliquoted or must be stored at -20℃ within 1 month or -80℃ within 3 months. Avoid repeated freeze thaw cycles.
- Samples should be brought to room temperature before starting the assay.
- Centrifuge to collect sample before use.
- Do not use lysis buffers with surfactants (e.g., SDS) or organic solvents (e.g., methanol) when preparing tissue homogenates, cell lysates, or similar samples.
- Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Certain recombinant proteins might go undetected if they do not match the coated or detection antibodies.

- For ELISA tests, plasma can be collected using typical anticoagulants like sodium or potassium EDTA and heparin salts. For biochemical assays or other experiments, evaluate any potential interference from these anticoagulants.

Reagent Preparation

- All reagents should be brought to room temperature before use.
- **Wash Buffer** Dilute 30 ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 750 ml of 1x Wash Buffer. If crystals have formed in the concentrate, warm it in a 40℃ water bath and mix gently until the crystals have completely dissolved.
- **Biotinylated Detection Ab** Calculate the required volume (100 µL per well) and prepare slightly more than needed. Centrifuge the concentrated biotinylated detection antibody at 800×g for 1 minute, then dilute the 100× concentrated version to a 1× working solution using biotinylated detection antibody diluent (mixing ratio 1:99 (ab:diluent)).
- **Standard** Centrifuge the Standard at 1000 × g for 1 minute. Reconstitute the Standard with 1 mL of Standard Diluent Buffer, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the Standard in the stock solution is 10 ng/mL. Please prepare 7 tubes containing 250 µL standard & sample diluent and use the Diluted Standard to produce a double dilution series according to the picture shown below. To mix each tube thoroughly before the next transfer, pipette the solution up and down several times. Set up 7 points of Diluted Standard such as **10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.32 ng/mL, 0.16 ng/mL,** and the last EP tubes with Standard Diluent is the Blank as 0 ng/mL. In order to guarantee the experimental results validity, please use the new Standard Solution for each experiment. When diluting the Standard from high concentration to low concentration, replace the pipette tip for each dilution. Note: the last tube is regarded as a Blank and do not pipette solution into it from the former tube.

10 ng/mL	Standard No.7	1 mL standard & sample diluent to working solution
5 ng/mL	Standard No.6	250 µL Standard No.7 + 250 µL standard & sample
2.5 ng/mL	Standard No.5	250 µL Standard No.6 + 250 µL standard & sample
1.25 ng/mL	Standard No.4	250 µL Standard No.5 + 250 µL standard & sample
0.63 ng/mL	Standard No.3	250 µL Standard No.4 + 250 µL standard & sample

0.32 ng/mL	Standard No.2	250 µL Standard No.3 + 250 µL standard & sample
0.16 ng/mL	Standard No.1	250 µL Standard No.2 + 250 µL standard & sample

- **HRP Conjugate** Calculate the required volume (100 µL per well) and prepare slightly more than needed. Centrifuge the concentrated HRP conjugate at 800×g for 1 minute, then dilute the 100× concentrated version to a 1× working solution using HRP conjugate diluent (mixing ratio 1:99 (ab:diluent)).

Assay Procedure

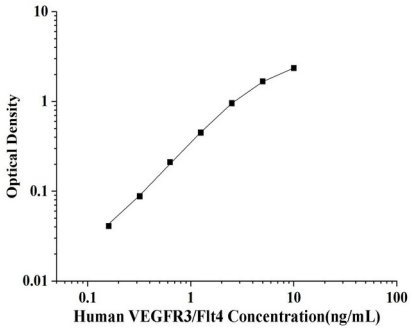
1. Determine wells for diluted standard, blank and sample. Add 100 µl each dilution of standard, blank and sample into the appropriate wells.
2. **Cover the plate with the sealer** provided in the kit. Incubate for 90 min at 37℃. Decant the solution from each well and pat it dry against clean absorbent paper.
3. Add 300 µl of wash buffer to each well. Soak for 0.5 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.
4. Add 100 µl of Biotinylated Detection Ab working solution to each well. **Cover the plate with a new sealer.** Incubate for 1 hour at 37°C.
5. Decant the solution from each well, repeat the wash process for 3 times as step 3.
6. Add 100 µl of HRP Conjugate working solution to each well. **Cover the plate with a new sealer.** Incubate for 30 min at 37℃.
7. Decant the solution from each well, repeat the wash process for 3 times as step 3.
8. Add 100 µl of Substrate Reagent to each well. **Cover the plate with a new sealer.** Incubate for about 15-20 min at 37°C. Protect the plate from light.
9. Add 50 µl of Stop Solution to each well.
10. Determine the Optical Density of each well at once with a micro plate reader set to 450 nm.

Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.



Summary

1. Prepare all reagents, samples and standards.
2. Add Standard or sample per well. Incubate for 90 min at 37°C. Wash the plate.
3. Add Biotinylated Ab. Incubate for 1 hour at 37°C. Wash the plate.
4. Add HRP Conjugate. Incubate for 30 min at 37°C. Wash the plate.
5. Add substrate reagent. Incubate for 15-20 minutes at 37°C.
6. Add stop solution and color develop.
7. Read the OD value within 30 minutes.

Shipping Direction - Temperature

Maintain temperature between 2°C to 8°C during transportation in unopened packaging.

Troubleshooting

Possible Case	Solution
High Background	
• Improper washing • Substrate was contaminated • Non-specific binding of antibody • Plate are not be sealing incompletely • Incorrect incubation temperature • Substrate exposed to light prior to use • Contaminated wash buffer	• Increasing duration of soaking steps • Replace. Substrate should be clean and avoid crossed contamination by using the sealer • Replace another purified antibody or blocking buffer • Make sure to follow the instruction strictly • Incubate at room temperature • Keep substrate in a dark place • Use a clean buffers and sterile filter
Weak Signal	
• Improper washing • Incorrect incubation temperature • Antibody are not enough • Reagent are contaminated • Pipette are not clean	• Increasing duration of soaking steps • Incubate at room temperature • Increase the concentration of the antibody • Use new one • Pipette should be clean
No Signal	
• Reagent are contaminated • Sample prepared incorrectly • Antibody are not enough • Wash buffer contains sodium azide • HRP was not added	• Use new one • Make sure the sample workable/dilution • Increase the antibody concentration • Use a new wash buffer and avoid sodium azide in it • Add HRP according to the instruction
Poor Precision	
• Imprecise/ inaccurate pipetting • Incomplete washing of the wells	• Check/ calibrate pipettes Make sure wells are washed adequately by filling the wells with wash buffer and all residual antibody solutions crossed well before washing.

This product is for research use only, not for use in diagnosis procedures.
It's highly recommended to read this instruction entirely before use.

For more information:
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