K-ASSAY®

KAMIYA BIOMEDICAL COMPANY

Human Endocrine Gland Vascular Endothelial Growth Factor (EG-VEGF) ELISA

For the quantitative determination of Human EG-VEGF in serum, plasma, cell culture supernatants, body fluid and tissue homogenate

Cat. No. KT-64085

For Research Use Only. Not for use in diagnostic procedures.

Rev. 64085

Product Information

Human Endocrine Gland Vascular Endothelial Growth Factor (EG-VEGF) ELISA

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INTENDED USE

This EG-VEGF ELISA kit is a 1.5 hour solid-phase ELISA designed for the quantitative determination of Human EG-VEGF. This ELISA kit is for research use only, not for therapeutic or diagnostic applications!

PRINCIPLE

This EG-VEGF ELISA kit applies the competitive enzyme immunoassay technique utilizing an anti-EG-VEGF antibody and an EG-VEGF-HRP conjugate. The assay sample and buffer are incubated together with EG-VEGF-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450 nm in a microplate reader. The intensity of the color is inversely proportional to the EG-VEGF concentration since EG-VEGF from samples and EG-VEGF-HRP conjugate compete for the anti-EG-VEGF antibody binding site. Since the number of sites is limited, as more sites are occupied by EG-VEGF from the sample, fewer sites are left to bind EG-VEGF-HRP conjugate. A calibration curve is plotted relating the intensity of the color (Optical Density) to the concentration of calibrators. The EG-VEGF concentration in each sample is interpolated from this calibration curve.

COMPONENTS

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

	Quantity
Microtiter Plate	96 wells
Calibrator 1 (0 pg/mL)	1
Calibrator 2 (100 pg/mL)	1
Calibrator 3 (250 pg/mL)	1
Calibrator 4 (500 pg/mL)	1
Calibrator 5 (1,000 pg/mL)	1
Calibrator 6 (2,500 pg/mL)	1
Enzyme Conjugate	1 x 6 mL
Substrate A	1 x 6 mL
Substrate B	1 x 6 mL
Stop Solution	1 x 6 mL
Wash Solution (100X concentrate)	1 x 10 mL
Balance Solution	1 x 3 mL

Note: The **Balance Solution** is used only when the sample is of **cell culture supernatants, body fluid and tissue homogenate**; if the sample is serum or plasma, then the balance solution is a superfluous reagent.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C. Centrifuge at approximately 1,000 x g (or 3,000 rpm) for 15 minutes. Collect serum and assay immediately or aliquot and store samples at -20°C or -80°C.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 x g (or 3,000 rpm) at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C.

Tissue homogenates

The preparation of tissue homogenates will vary depending upon the tissue type. (For this assay, tissues were rinsed in ice-cold PBS (0.02 mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed 300-500 mg before homogenization.) Mince tissues into small pieces and homogenize in 500 µL of PBS with a glass homogenizer on ice. The resulting suspension should then be subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates should be centrifugated for 15 minutes at 1,500 x g (or 5,000 rpm). Collect the supernate and assay immediately or aliquot and store samples at -20°C or -80°C.

Cell lysates

Cells should be lysed according to the following directions.

1. Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
2. Wash cells three times in PBS. For the collection of samples, the amount of cells should be no less than 10^8 in 200 µL PBS
3. Resuspend cells in PBS and subject them to ultrasonication 3 times. Alternatively, freeze cells at -20°C.Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.
4. Centrifuge at 1,000 x g (or 3,000 rpm) for 15 minutes at 4°C to remove cellular debris.
5. Assay immediately or store samples at -20°C or -80°C.

Cell culture supernatants and other body fluids

Centrifuge cell culture media at 1,000 x g (or 3,000 rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20°C or -80°C.

NOTE:

1. Samples should be aliquoted and must be stored at -20°C (less than 3 months) or -80°C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 4°C. Avoid repeated freeze-thaw cycles.
2. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
3. Samples containing a visible precipitate must be clarified prior to use in the assay. Care should be taken to minimize hemolysis. Do not use grossly hemolyzed or lipemic specimens.
4. Do not use heat-treated specimens.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision pipettors and disposable tips to deliver 10-1,000 µL. A multi-channel pipette is desirable for large assays.

2. 100 mL and 1 liter graduated cylinders.
3. Distilled or deionized water.
4. Tubes to prepare sample dilutions.
5. Absorbent paper.
6. Microplate reader which is capable of measuring absorbance at 450 nm.
7. Centrifuge which is capable of 3,000 x g.
8. Microplate washer or washing bottle.
9. Incubator (37°C).
10. Data analysis and graphing software.

SAMPLE PREPARATION

1. **Kamiya Biomedical Company** is responsible only for the kit itself, and not for the samples consumed during the assay. The user should calculate the possible amount of the samples used throughout the entire test. Please reserve a sufficient amount of samples in advance.
2. Predict the concentration of samples before assaying. If values for these are not within the range of the calibration curve, **users must determine the optimal sample dilutions for their particular experiments.** We suggest pre-experimenting with neat (undiluted) samples, 1:2 or 1:4 dilutions. Avoid diluting your samples more than 1:10 as it would exceed the dilution limit set for this kit.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Owing to the possibility of mismatching between antigens from other sources and the antibody used in our kits (e.g. antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
5. Influenced by various factors including cell viability, cell number and sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Fresh samples that have not been in long term storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature before use.
2. **Samples** – Predict the concentration before assaying. If concentrations are unknown or not within the detection range, a preliminary experiment is recommended to determine the optimal dilution. PBS (pH 7.0-7.2) or 0.9% physiological saline can be used as dilution buffer.
3. **Wash Solution** - Dilute 10 mL of **Wash Solution concentrate (100x)** with 990 mL of deionized or distilled water to prepare 1,000 mL of **Wash Solution (1x)**. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The 1x wash solution is stable for 2 weeks at 4°C.
4. Do not dilute the other components which are ready-to-use.

ASSAY PROCEDURE

Please read the **Reagent Preparation** section before starting the assay procedure**. It is recommended that all Calibrators and Samples be assayed in duplicate**. It is strongly recommended to do a preliminary experiment before measuring all samples.

1. Secure the desired number of coated wells in the holder then add 100 µL of **Calibrators** (Shake the bottle of each calibrator gently by hand and pipette up and down the solution 3 times before adding) or **Samples,** to the appropriate well. Add 100 µL of PBS (pH 7.0-7.2) into the blank control well.
2. Dispense 10 µL of **Balance Solution** into 100 µL of **samples only**, mix well. (**NOTE:** This step is required **ONLY** when the sample is of cell culture supernatants, body fluid and tissue homogenates; if the sample is either serum or plasma, then this step should be skipped.)
3. Add 50 µL of **Enzyme Conjugate** to each well **(NOT the blank control well).** Mix well. Mixing well in this step is important. Cover and incubate the plate for 1 hour at 37°C.
4. Wash the microtiter plate using one of the specified methods indicated below:
   - **Manual Washing** – Remove the incubation mixture by aspirating the contents of the plate into a sink or proper waste container. Fill each well completely with **1x Wash Solution** and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of **FIVE** washes. Take care not to scratch the surface of the wells.
   - **Automated Washing** - Wash the plate **FIVE** times with diluted wash solution (350-400 µL/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
5. After washing, invert the plate and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. Complete removal of liquid at each step is essential to good performance.
6. Add 50 µL **Substrate A** and 50 µL **Substrate B** to each well including the blank control well, subsequently. Cover and incubate the plate for 15-20 minutes at 37°C. (Avoid sunlight).
7. Add 50 µL of **Stop Solution** to each well including the blank control well. Mix well.
8. Determine the Optical Density (O.D.) at 450 nm using a microplate reader immediately.

CALCULATION OF RESULTS

1. The calibration curve is used to determine the amount of samples.
2. First, average the duplicate readings for each calibrator and sample. All O.D. values are subtracted by the mean value of the blank control before result interpretation. **DO NOT** subtract the O.D. of calibrator zero.
3. Construct a calibration curve by plotting the concentration on the horizontal (X) axis against the average O.D. for each calibrator on the vertical (Y) axis. Then draw a best fit curve using graph paper or statistical software to generate a linear regression, four parameter logistic (4-PL) curve-fit or curvilinear regression of second degree. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.
4. Calculate the concentration of samples corresponding to the mean absorbance from the calibration curve.

NOTE:

- Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variations in the results. Each user should obtain their own calibration curve.
- If the samples have been diluted, the concentration read from calibration curve must be multiplied by the dilution factor.
- If specimens generate values higher than the highest
- calibrator, dilute the specimens and repeat the assay.

CERTIFICATE OF ANALYSIS

1. Same lot CV%: 4.4, 5.6
2. Different lot CV%: 6.6, 7.9
3. Spike Recovery: 94 - 103%
4. Linearity:

	Range %
1:1	96 - 101
1:2	93 - 107
1:4	92 - 100
1:8	96 - 108

5. **Sensitivity: The sensitivity of this assay is 1.0 pg/mL.**
6. S**pecificity:** This assay has a high sensitivity and excellent specificity for the detection of EG-VEGF . No significant cross-reactivity or interference between EG-VEGF and analogues was observed.

NOTE: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between EG-VEGF and all of its analogues, therefore, cross reaction may still exist in some cases.

SAFETY NOTES

1. This kit contains a small amount of 3,3',5,5'-Tetramethylbenzidine (TMB) in **Substrate B**. TMB is non-carcinogenic but it is hazardous in case of skin contact, eye contact, ingestion and inhalation. In case of contact, rinse the affected area with plenty of water.
2. The **Stop Solution** provided with this kit is an acid solution. Wear protective gloves, clothing, and face protection.
3. Care should be taken when handling the **Calibrator** because of the known and unknown effects of it.
4. Care should also be taken with the other kit reagents and samples to avoid contact with the skin or eyes. In the case of contact, wash immediately with water.
5. Do not pipette by mouth.
6. Avoid generating aerosols.
7. Waste must be disposed of in accordance with federal, state and local environmental control regulations.
8. All blood components and biological materials should be handled as potentially hazardous. Decontaminate and dispose of specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is to autoclave for a minimum of 1 hour at 121.5°C.

QUALITY CONTROL

1. **It is recommended that all calibrators, controls and samples be run in duplicate**. Calibrators and samples must be assayed at the same time.
2. The coefficient of determination of the calibration curve should be ≥0.95 and the highest O.D. should be more than 1.0.
3. Cover or cap all kit components and store at 4°C when not in use.

4. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 4°C to maintain plate integrity.
5. Samples should be collected in pyrogen/endotoxin-free tubes.
6. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
7. When possible, avoid using badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Do not mix or interchange different reagent lots from various kit lots.
10. Do not use reagents after the kit expiration date.
11. Read absorbance immediately after adding the stop solution.
12. Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
13. Because **TMB** is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB and metal, otherwise color may develop.

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