

Human VEGF ELISA Kit

to determine Human VEGF in Serum, Blood Plasma, Saliva, Urine, Tissue
Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT11700EAYQ



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INTENDED USE

This kit is used to determine the Human VEGF in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions. For *in vitro* use only.

MANUAL VERSION 1.02

ASSAY PRINCIPLE

Human VEGF ELISA Kit employs a two-site sandwich ELISA to quantitate VEGF in samples. An antibody specific for VEGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any VEGF present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for VEGF is added to the wells. After washing, proprietary Streptavidin-HRP conjugates is added to the wells. Following a wash to remove any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of VEGF bound in the initial step. The color development is stopped and the intensity of the color is measured

CHARACTERISTICS

- This Kit allows for the determination of Human VEGF concentrations in serum, cell culture supernates and other biological fluids..
- Detection range: 31.25 pg/mL- 2000 pg/mL
- The minimum detectable dose (MDD) of Human VEGF is typically less than 16 pg/mL.
- Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) < 10%.
- Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%) < 10%.
- To assess linearity of the assay, samples containing and/or spiked with high concentrations of Human VEGF were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the

assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

- Human VEGF ELISA Kit has high sensitivity and excellent specificity for detection of Human VEGF. No significant cross-reactivity or interference between Human VEGF analogues was observed.

PRODUCT INFORMATION

MATERIALS SUPPLIED AND STORAGE CONDITIONS

Store kit reagents at 2-8 °C for 12 months.

Components
Human VEGF microplate, 96T
Human VEGF standard, lyophilized
Human VEGF detection antibody, 100x
Sample diluent, 5x liquid
Streptavidin-HRP, 100x liquid
Stop solution
Wash buffer, 20x
Plate Covers
Booklet

¹Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator
2. Microplate reader
3. Precision pipettes and tips

4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell culture supernatants - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored at -20 °C to avoid loss of bioactive VEGF. If samples are to be used within 24 hours, they may be stored at 2 to 8 °C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Note: Samples should be centrifuged adequately and no hemolysis or granule was allowed.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

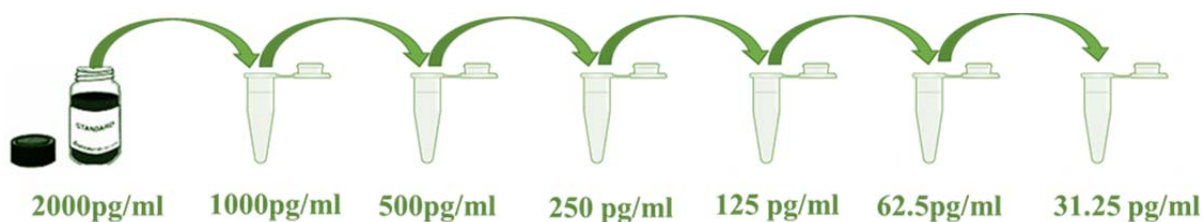
Wash buffer Pour entire contents (50 ml) of the Wash buffer (20×) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2 to 25 °C. Please note that Wash buffer (1×) is stable for 30 days.-

Sample Diluent/ Assay buffer - Pour the entire contents (7 ml) of the Diluent (5×) into a clean 100 ml graduated cylinder. Bring to final volume of 35 ml with distilled water. Mix gently to avoid foaming. Store at 2 to 8 °C. Please note that the Diluent (1×) is stable for 30 days.

HRP substrate - The reagents should be ready within 15 minutes of use. Protect from light. 100 µL of the solution is required per well.

Standard - Reconstitute the Human VEGF standard in 1mL of Sample Diluent for a concentration of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µl of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (2000 pg/mL). Sample Diluent serves as the zero standard (0 pg/mL).



Human VEGF detection antibody - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated detection antibody solution with Assay buffer in a clean plastic tube as needed according to the standards and samples. Detection antibody should be used within 30 minutes after dilution.

Streptavidin-HRP - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated Streptavidin-HRP with Assay buffer in a clean plastic tube as needed according to the standards and samples. Streptavidin-HRP should be used within 30 minutes after dilution.

Sample dilution - If your samples need to be diluted, Sample diluent is used for dilution of serum/plasma samples, while cell culture medium is used for dilution of cell culture supernatants.

ASSAY PROCEDURE

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 μ L of diluted standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.
4. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash buffer (250 μ L) using a squirt bottle, multi-channel pipette, manifold dispenser, or automatic washer. 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.

5. Add 100 μ L of diluted Human VEGF detection antibody to each well. Cover with a new adhesive strip. Incubate for 1 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μ L of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash process for five times as in step 4.
9. Add 100 μ L of HRP substrate solution to each well. Incubate for 15 minutes at room temperature. Protect from light.
10. Add 50 μ L of Stop solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration for each standard on the x-axis and draw a best fit curve through the points on the

graph. The data may be linearized by plotting the log of the Human VEGF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

PRECAUTIONS

1. Do not mix or substitute reagents with those from other lots or sources.
2. This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
3. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
7. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
8. Chromogen Solution is easily contaminated. If bluish prior to use, do not use.
9. Stop Solution should be added to the plate in the same order as the

Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Chromogen solution.

10. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
11. All samples should be disposed of in a manner that will inactivate viruses.
12. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%.
The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

VALIDITY & STORAGE: 12 Months for unopened kit. Storage 2-8°C