

Life Tech E Series Mouse VEGF ELISA Kit

to determine Mouse VEGF in Serum, Blood Plasma, Saliva, Urine, Tissue

Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT6107TEKKBA



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INTENDED USE

This kit is used to determine VEGF (Vascular endothelial growth factor) in mouse blood plasma, saliva, urine, tissue liquid samples or related biological solutions.

For *in vitro* use only.

MANUAL VERSION 1.02

INTRODUCTION

ASSAY PRINCIPLE

Mouse 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 Mouse VEGF concentrations in Mouse serum, cell culture supernates and other biological fluids.
- Detection range: 15.6 pg/mL- 1000 pg/mL
- The minimum detectable dose (MDD) of Mouse VEGF is typically less than 8 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 Mouse 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.

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

PRODUCT INFORMATION

MATERIALS SUPPLIED AND STORAGE CONDITIONS

Store kit reagents at 2-8 °C for 12 months. Immediately after use remaining reagents should be returned to cold storage at 4 °C.

Mouse VEGF microplate: 96 well polystyrene microplates (12 strips of 8 wells) coated with the antibody specific for Mouse VEGF.

Mouse VEGF standard: Mouse VEGF in a buffered protein base with preservatives; lyophilized.

Sample diluent: Diluent solution for reconstituted standard and samples. 5× liquid. **Mouse VEGF detection antibody:** Biotin-conjugated Mouse VEGF detection antibody. 100× liquid.

Streptavidin-HRP: 100× liquid.

Assay buffer: Diluent solution for detection antibodies and Streptavidin-HRP. 5× liquid.

HRP substrate: TMB (Tetramethyl-benzidine) solution.

Stop solution: 2N sulfuric acid.

Wash buffer: PBS with 1% Tween-20; 20× liquid.

Plate covers.

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. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.

- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder

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.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for two hours at

room temperature or overnight at 4 °C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection.

Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

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

REAGENT PREPARATION

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.

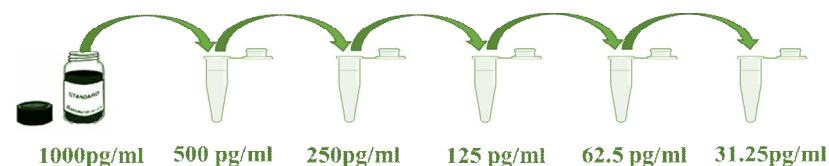
Standard - Reconstitute the Mouse VEGF standard in 1mL of Sample Diluent for a concentration of 1000 pg/mL. Allow the standard to sit for a minimum of 15

minutes with gentle agitation prior to making dilutions.

Add 500 µL of Standard Diluent Buffer to each of 6 tubes labeled 500, 250, 125, 62.5, 31.25 and 15.6 pg/mL of Mouse VEGF standard just as below.

ASSAY PROCEDURE

1. Dilution of standard solution: This kit contains a standard of known concentration, which could be diluted in small tubes by the end-user by following the instruction in the table below:



Note: If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

2. Prepare all reagents and working standards as directed in the previous sections.
3. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
4. Add 100 µL 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.
5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash buffer (250 µL) 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.

6. Add 100 µL of diluted Mouse VEGF detection antibody to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.

7. Repeat the aspiration/wash as in step 4.

8. Add 100 µL 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.

9. Repeat the aspiration/wash process for five times as in step 4.

10. Add 100 µL of HRP substrate solution to each well. Incubate for 15 minutes at room temperature. Protect from light.

11. Add 50 µL 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.

12. 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 Mouse VEGF concentration 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 (for e.g. it is 5 in this case).

TYPICAL DATA

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

PRECAUTIONS

1 Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.

Stop Solution contains 2 N Sulfuric Acid (H₂SO₄) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

VALIDITY & STORAGE: One Year from date of supply (for unopened kit).

2-8°C