Mouse VEGF ELISA Kit

Mouse VEGF ELISA Kit is an Enzyme Immunoassay kit for the quantification of Mouse VEGF in serum, plasma and cell culture supernatants.

Catalog number: ARG83472

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

This gene product is a member of the PDGF/VEGF growth factor family. It is a mitogen that specifically acts on endothelial cells and has various effects, including mediating increased vascular permeability, inducing angiogenesis, vasculogenesis, endothelial cell growth, promoting cell migration, and inhibiting apoptosis. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. Also, alternative translation initiation from non-AUG (CUG) and AUG start sites in some transcript variants, give rise to additional isoforms. [provided by RefSeq, Jul 2008]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Mouse VEGF has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any VEGF present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Mouse VEGF is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Mouse VEGF bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ± 2nm. The concentration of Mouse VEGF in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 100X Antibody Conjugate concentrate at ≤ -20°C. Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C.
Standard (Lyophilized)	3 X 1 ng/vial	4°C
Standard / Sample Diluent Buffer	20 mL (Ready to use)	4°C
100X Antibody Conjugate Concentrate	1 vial (120 µL)	-20°C
40X HRP-Streptavidin Solution	1 vial (300 µL)	4°C
Antibody Diluent Buffer	30 mL (Ready to use)	4°C
20X Wash Buffer	45 mL	4°C
TMB Substrate	12 mL (Ready to use)	4°C (Protect from light)
Stop Solution	12 mL (Ready to use)	4°C
Plate Sealers	3 adhesive strips	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- Automated microplate washer (optional)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store 100X Antibody Conjugate concentrate at ≤ -20°C. Store other component at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

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 for 10 min at 1500 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

- Do not use haemolytic, icteric or lipaemic specimens.
- Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash Buffer:** Dilute **20X** Wash Buffer into 1X distilled water to yield 1X Wash Buffer (E.g., add 25 mL of **20X Wash Buffer** into 475 mL of distilled water to a final volume of 500 mL).
  The diluted 1X Wash Buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Antibody Conjugate:** 20 minutes before use, dilute **100X** Antibody Conjugate concentrate into **Antibody Diluent Buffer** to yield 1X Antibody Conjugate.
- **1X HRP-Streptavidin Solution:** 20 minutes before use, dilute **40X** HRP-Streptavidin concentrate into **Antibody Diluent Buffer** to yield 1X HRP-Streptavidin Solution. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Sample:** Diluent serum and plasma samples with equal volume of **Standard / Sample Diluent Buffer** before assay (1:1, dilution factor=2). If the initial assay found samples contain proteins higher than the highest standard, the samples can be diluted with Standard / Sample Diluent Buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. Cell culture supernatants could be assayed directly. (It is recommended to do pre-test to determine the suitable dilution factor).
- **Standard:** Centrifuge the un-reconstituted standard at 6000 x g for 1 minute to bring down the material prior to open the vial. Add **1 ml** of **Standard / Sample Diluent Buffer** to a Standard vial to make the high standard concentration of 1000 pg/mL and brief vortex for few seconds

(Do not induce foaming) and allow it to sit for 15 minutes. Make sure the standard is dissolved completely before making serial dilutions. The Standard / Sample Diluent Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard / Sample Diluent Buffer. Diluted the standard as below:

Standard tube	VEGF (pg/mL)	Standard / Sample Diluent Buffer (µL)	Standard (µL)
S1	1000	0	1000 (1000 pg/mL Standard Stock)
S2	500	500	500 of S1
S3	250	500	500 of S2
S4	125	500	500 of S3
S5	62.5	500	500 of S4
S6	31.25	500	500 of S5
S7	15.625	500	500 of S6
S0	0	500	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates.

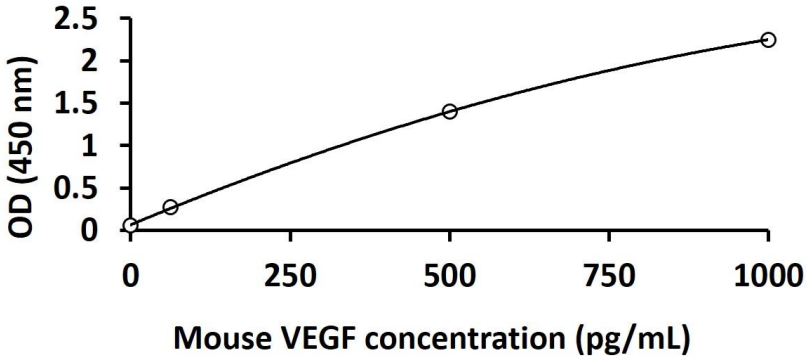
1. Add **100 µL** of **diluted samples** or **each diluted Standard** into respective wells of the 96-well plate.
2. Cover the plate and incubate for **2 hours** at **room temperature**.
3. Aspirate each well and wash, repeating the process 4 time for a **total 5 washes.** Wash by filling each well with **1X Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **1X Antibody Conjugate** to each well.
5. Cover the plate and incubate for **1 hour** at **room temperature**.
6. Aspirate each well and **wash** plate **as step 3**.
7. Add **100 µL** of **1X HRP-Streptavidin Solution** to each well.
8. Cover the plate and incubate for **30 mins** at **room temperature** in the dark.
9. Aspirate each well and **wash** plate **as step 3**.
10. Add **100 µL** of **TMB Substrate** in each well.
11. Incubate for **15-25 mins** at **room temperature** in the dark.
12. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
13. Read the absorbance with a plate reader at **O.D. 450 nm.** It is recommended reading the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Mouse VEGF ELISA Kit. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

7.8 pg/ml

Assay Range

15.625 - 1000 pg/mL