arigoQIK™ Mouse VEGF ELISA Development Kit

ARG88009 arigoQIK™ Mouse VEGF ELISA Development Kit is designed for the development of sandwich ELISA to measure Mouse VEGF in serum, plasma, cell culture supernatants.

Catalog number: ARG88009

Package: 1 kit (5 plates)
(15 plates)

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INTRODUCTION

This gene is a member of the PDGF/VEGF growth factor family. It encodes a heparin-binding protein, which exists as a disulfide-linked homodimer. This growth factor induces proliferation and migration of vascular endothelial cells, and is essential for both physiological and pathological angiogenesis. Disruption of this gene in mice resulted in abnormal embryonic blood vessel formation. This gene is upregulated in many known tumors and its expression is correlated with tumor stage and progression. Elevated levels of this protein are found in patients with POEMS syndrome, also known as Crow-Fukase syndrome. Allelic variants of this gene have been associated with microvascular complications of diabetes 1 (MVCD1) and atherosclerosis. Alternatively spliced transcript variants encoding different isoforms have been described. There is also evidence for alternative translation initiation from upstream non-AUG (CUG) codons resulting in additional isoforms. A recent study showed that a C-terminally extended isoform is produced by use of an alternative in-frame translation termination codon via a stop codon readthrough mechanism, and that this isoform is antiangiogenic. Expression of some isoforms derived from the AUG start codon is regulated by a small upstream open reading frame, which is located within an internal ribosome entry site. The levels of VEGF are increased during infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), thus promoting inflammation by facilitating recruitment of inflammatory cells, and by increasing the level of angiopoietin II (Ang II), one of two products of the SARS-CoV-2 binding target, angiotensin-converting enzyme 2 (ACE2). In turn, Ang II facilitates the elevation of VEGF, thus forming

a vicious cycle in the release of inflammatory cytokines. [provided by RefSeq, Jun 2020]

PRINCIPLE OF THE ASSAY

This pair employs the quantitative sandwich enzyme immunoassay technique. Coating specific VEGF antibody on 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 VEGF Detection is added to each well and incubate. Following a washing to remove unbound substances, HRP-Streptavidin Solution is added to each microplate well and incubated. After washing away any unbound antibody, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of GM-CSF 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. The concentration of 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 Human GM-CSF Detection at ≤ -20°C.

Store other components at 2-8°C at all times. Use the kit before expiration date.

Component	Package (5 plates)	Package (15 plates)	Storage information
100X Mouse VEGF Capture Antibody	1 vial	3 vial	4°C
Mouse VEGF Standard	1 vial	3 vial	4°C
100X Mouse VEGF Detection Antibody	1 vial	3 vial	≤ -20°C
100X HRP-Streptavidin Solution	1 vial	3 vial	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Phosphate buffered saline (PBS)
- Wash buffer
- Antibody Diluent Buffer, Standard / Sample Diluent Buffer
- Blocking Buffer
- Substrate Solution, Stop Solution
- Deionized or distilled water
- 96-well plate
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microplate reader capable of reading at 450 nm
- Microtiter plate washer (optional)
- For commercial reagents required for arigoQIK™ ELISA Development Kit, please refer ARG83524 Integral Reagent Kit (ELISA Development Kit)

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

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:

a) Do not use haemolytic, icteric or lipaemic specimens.
b) Samples containing sodium azide should not be used in the assay.
c) Avoid disturbing the white buffy layer when collection serum / plasma sample.

REAGENT PREPARATION

- **Phosphate buffered saline (PBS)**: Prepared with 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.5 mM KH_2PO_4. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.
- **10X Wash Buffer**: Add Tween-20 to the previously prepared PBS to achieve a final concentration of 0.5%. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.

  Dilute 10X Wash Buffer with distilled water to yield 1X Wash Buffer. The diluted 1X Wash Buffer is stable for 4 weeks at 2°C to 8°C.
- **Standard / Sample Diluent Buffer**: Add BSA and Tween-20 to the previously prepared PBS to achieve a final concentration of 1% BSA and 0.05% Tween-20. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.
- **Antibody Diluent Buffer**: Add BSA and Tween-20 to the previously prepared PBS to achieve a final concentration of 1% BSA and 0.1% Tween-20. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.
- **Blocking Buffer**: Add BSA and Tween-20 to the previously prepared PBS to achieve a final concentration of 1% BSA and 0.05% Tween-20. The solution is adjusted to a pH range of 7.2 to 7.4 and is filtered using a 0.2 µm filter.

- **1X Mouse VEGF Capture**: It is recommended to prepare this reagent immediately prior to use and use it within 15 min after preparation. Dilute 100X Mouse VEGF Capture with prepared **PBS** previously, to yield 1X Mouse VEGF Capture. The diluted 1X Mouse VEGF Capture is stable for 8 weeks at 2-8°C. For long-term storage, aliquot and store at-20°C to-70 °C.
- **1X Mouse VEGF Detection**: It is recommended to prepare this reagent immediately prior to use and use it within 15 min after preparation. Dilute 100X Mouse VEGF Detection with prepare **Antibody Diluent Buffer** previously, to yield 1X Mouse VEGF Detection. The diluted 1X Mouse VEGF Detection is stable for 8 weeks at 2-8°C. For long-term storage, aliquot and store at-20°C to-70 °C.
- **1X HRP-Streptavidin Solution**: It is recommended to prepare this reagent immediately prior to use and use it within 15 min after preparation. Dilute 100X HRP-Streptavidin Solution with prepare **Antibody Diluent Buffer** previously, to yield 1X HRP-Streptavidin Solution. The diluted 1X HRP-Streptavidin Solution is stable for 4 weeks at 2-8°C. Do NOT freeze 1X HRP-Streptavidin Solution.
- **Sample**: 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.

Standard: Centrifuge the un-reconstituted standard at 6000 x g for 1 minute to bring down the material prior to open the vial. Reconstituted Standard as label on the Standard vial. The reconstituted Standard stock concentration would be 30000 pg/ml. Vortex for few seconds (Do not induce foaming) and allow it to sit for 15 minutes as stock. The Standard stock is stable for 4 weeks at 2-8°C. For long-term storage, aliquot and store at-20°C to-70 °C.

- 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 stock (µL)
Pre1	3000	900	100 (30000 pg/ml Standard Stock)
S1	1500	500	500 (3000 pg/ml Pre1)
S2	750	500	500 of S1
S3	375	500	500 of S2
S4	188	500	500 of S3
S5	94	500	500 of S4
S6	46.88	500	500 of S5
S7	23.44	500	500 of S6
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature before use.

1. Add **100 µL** of **1X Mouse VEGF Capture** into respective well.
2. Cover the plate and incubate for **16-20 hours** at 4°C.
3. Remove the cover and discard the liquid in the wells.
4. Add **250 µl** of **Blocking Buffer** to each well.
5. Cover the plate and incubate for **2 hours** at room temperature.
6. Remove the cover and discard the liquid in the wells.
7. Add **100 µL** of **samples** and **each diluted Standard** into respective well.
8. Add **50 µL** of **1X Mouse VEGF Detection** into each well.
9. Cover the plate and incubate for **1 hour** at room temperature on a microplate shaker set at 500 rpm.
10. 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 auto-washer. 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.
11. Add **100 µL** of **1X HRP-Streptavidin Solution** into each well.
12. Cover the plate and incubate for **30 min** at room temperature in the dark on a microplate shaker set at 500 rpm.
13. Aspirate each well and **wash** plate **as step 10**.
14. Add **100 µL** of **TMB Substrate** in each well.
15. Incubate for **5-30 mins** at room temperature in the dark.
16. Add **100 µL** of **Stop Solution** to each well to stop the reaction.

17. Read the absorbance with a plate reader at O.D. 450 nm 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.