Murine VEGF Pre-Coated ELISA Kit

Catalog Number: BGK00731

ELISA kit for the accurate quantitation of Murine VEGF concentrations in cell culture supernates, serum, plasma, and other biological fluids.

For research use only. Not for use in diagnostic procedures. Please read the datasheet in its entirety before using this product.

Description

VEGF is a potent growth and angiogenic cytokine. It stimulates proliferation and survival of endothelial cells, and promotes angiogenesis and vascular permeability. Expressed in vascularized tissues, VEGF plays a prominent role in normal and pathological angiogenesis. Substantial evidence implicates VEGF in the induction of tumor metastasis and intra-ocular neovascular syndromes. VEGF signals through the three receptors; fms-like tyrosine kinase (flt-1), KDR gene product (the murine homolog of KDR is the flk-1 gene product) and the flt4 gene product.

The BioGems Murine VEGF Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Murine VEGF with a 96-well strip plate that is pre-coated with rat monoclonal antibody specific for VEGF. It contains sf21-expressed recombinant Murine VEGF. The detection antibody is a biotinylated goat polyclonal antibody specific for VEGF. The kit is analytically validated with ready to use reagents.

Precaution

For Research use only. Not for use in diagnostic or therapeutic procedures.

Please read the instructions fully prior to beginning the use of the assay kit.

Do not substitute reagents from other sources.

Variations or modifications of the described protocol or the use of other reagents can result in a reduction of product performance.

Storage and Stability

Upon receipt store the unopened kit at 4°C for 6 months or -20°C for 12 months. Avoid repeated freeze/thaw cycles.

Materials Provided

Description	Quantity	Volume
Anti-Murine VEGF Pre-coated 96-well strip microplate	1	12 strips of 8 wells
Murine VEGF Standard	2	10 ng vial
Murine VEGF Biotinylated antibody (100x)	1	130 µL
Avidin-Biotin-Peroxidase Complex (100x)	1	130 µL
Sample Diluent	1	30mL
Antibody Diluent	1	12mL
Avidin-Biotin-Peroxidase Diluent	1	12mL
Wash Buffer Concentrate (25X)	1	20mL
Color Developing Reagent	1	10mL
Stop Solution	1	10mL
Plate Sealers	4	Each

Required Materials not supplied

Microplate Reader capable of reading absorbance at 450nm.

Automated plate washer (optional)

Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

Deionized or distilled water.

500mL graduated cylinders.

Test tubes for dilution.

Reagent Preparation

Bring all reagents to room temperature prior to use.

Wash buffer- Dilute the supplied Wash Buffer Concentrate with 480ml of deionized or distilled water.

Biotinylated Anti-Murine VEGF antibody- It is recommended to prepare this reagent immediately prior to use by diluting the Murine VEGF Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µL by adding 1 µL of Biotinylated antibody (100x) to 99 µL of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

Avidin-Biotin-Peroxidase Complex- It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µL by adding 1 µL of Avidin-Biotin-Peroxidase Complex (100x) to 99 µL of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

Murine VEGF Standard- It is recommended that the standards be prepared no greater than 2 hours prior to performing the experiment. Use one 10ng vial of lyophilized Murine VEGF standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10,000 pg/mL using 1mL of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

Dilution of Murine VEGF Standard-

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –1,000 pg/mL, #2 –500 pg/mL, #3 – v pg/mL, #4 – 125 pg/mL, #5 – 62.5 pg/mL, #6 – 31.2 pg/mL, #7 – 15.6 pg/mL, #8 – 0.0 (Blank).
2. To generate standard #1, add 100 µL of the reconstituted standard stock solution of 10,000 pg/mL and 900 µL of sample diluent to tube #1 for a final volume of 1,000 µL. Mix thoroughly.
3. Add 300 µL of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µL of standard #1 from tube #1 to tube #2 for a final volume of 600 µL. Mix thoroughly.
5. To generate standard #3, add 300 µL of standard #2 from tube #2 to tube #3 for a final volume of 600 µL. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank standard to be used with every experiment.

Microplate- The included microplate is provided ready to use and does not require additional rinsing or blocking. The unused well strips should be sealed and stored in the original packaging.

Sample Preparation

The recommended sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Cell Culture supernatants- Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.

Serum- Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.

Plasma- Collect plasma using heparin or EDTA as an anticoagulant. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µL of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay procedure

It is recommended that all reagents and materials be equilibrated to room temperature prior to the experiment.

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µL of the standard, samples, or control per well. At least two replicates of each standard, sample, or control is recommended
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µL of the prepared 1x Biotinylated Anti-Human Growth Hormone antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 2 more additional times.
9. Add 100 µL of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 4 more additional times.
11. Add 90 µL of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µL of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Calculation of results

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

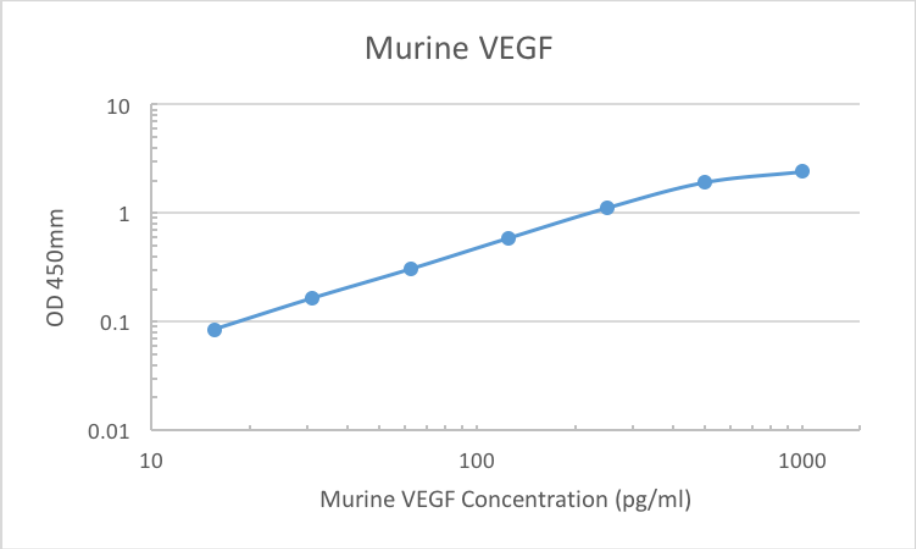
For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Typical Data

Standard #	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**
Standard Concentration (pg/mL)	0	15.6	31.2	62.5	125	250	500	1000
OD_{450}	0.008	0.085	0.165	0.307	0.592	1.122	1.934	2.432

Typical Standard Curve

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



Sensitivity

The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

Specifications

Range- 15.6 pg/mL- 1,000 pg/mL

Sensitivity- <2 pg/ml

Specificity- Natural and recombinant Murine VEGF (UniProt ID: Q00731)

Cross-Reactivity- No detectable cross-reactivity with other relevant proteins

Reproducibility

	Intra-Assay			Inter-Assay		
Sample ID	1	2	3	1	2	3
n=	16	16	16	24	24	24
Mean Measured Concentration (pg/mL)	126	341	580	212	475	624
Standard Deviation	4.41	13.64	28.42	10.6	30.4	46.8
Consistency (%CV)	3.5	4	4.9	5	6.4	7.5