GSI Equine VEGF ELISA Kit-DataSheet

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF) or vasculotropin, is a homodimeric 34 - 42 kDa, heparin-binding glycoprotein with potent angiogenic, mitogenic and vascular permeability-enhancing activities specific for endothelial cells. VEGF is a sub-family of platelet-derived growth factor family of cystine-knot growth factors. The most important member is VEGF-A. Other members are Placenta growth factor (PlGF), VEGF-B, VEGF-C and VEGF-D. The amino acid sequence of VEGF exhibits primary structural, as well as limited amino acid sequence, homology with that of the A and B chains of PDGF. All eight cysteine residues involved in intra- and inter-chain disulfide bonds are conserved among these growth factors. Two receptor tyrosine kinases have been described as putative VEGF receptors. Flt-1 (fms-like tyrosine kinase), and KDR (kinase-insert-domain-containing receptor) proteins have been shown to bind VEGF with high affinity (1). VEGF acts directly on the endothelium and does not degranulate mast cells. It promotes extravasation of plasma fibrinogen, leading to fibrin deposition which alters the tumor extracellular matrix. The modified extracellular matrix subsequently promotes the migration of macrophages, fibroblasts and endothelial cells. VEGF plays important roles in inflammation and during normal and pathological angiogenesis, a process that is associated with wound healing, embryonic development, and growth and metastasis of solid tumors. Elevated levels of VEGF have been reported in synovial fluids of rheumatoid arthritis patients and in sera from cancer patients (2, 3).

References

1. Katherine H, et al. (2007). Cell Signal. 19 (10): 2003
2. Amo, Y, et al. (2004). Br J Dermato. 150 (1): 160
3. Bergers G, et al. (2008). Nat. Rev. Cancer 8 (8): 592

PRINCIPLE OF THE ASSAY

This is a shorter ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance and the sensitivity of the assays. An antibody specific for equine 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 washing away any unbound substances, a detection antibody specific for equine VEGF is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash 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.

This package insert must be read in its entirety before using this product.

Storage

Store the standard, detection antibody at 4°C ~ -20°C, 10 x reagent diluents and the antibody pre-coated plate at -20°C, and store the rest of the kit at 4°C. The kit can be used in 3 months.

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	20 x Wash Buffer	1	Stop Solution	1
Detection Agent	1	10 x Reagent Diluent	1	DataSheet/Manual	1
Standard	3			96-well plate sheet	1

Bring all reagents to room temperature before use.

1 x **96-well Plate precoated with Equine VEGF capture antibody-**Store at -20°C upon received.

Equine VEGF Detection Antibody– The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of 1 x Reagent Diluent to the antibody vial, vortex 20 sec and allow it to sit for 5 min. Take 200 µL of detection antibody to 9.8 mL Reagent Diluent if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

Equine VEGF Standard (3 vials) – The lyophilized Equine VEGF Standard has a total of 3 vials. Each vial contains the standard sufficient for a 96-well plate. The non-reconstituted standard can be stored at 4°C ~ -20°C for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the tube. Add 500 µL of 1 x Reagent Diluent to a standard vial to make the high standard concentration of 2200 pg /ml. Vortex 20 sec and allow it to sit for 5 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in Reagent Diluent, vortex 20 sec for each of dilution step. Store the rest of the standard at -20°C.

Detection Agent (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. If the volume is less than 50 µL, add 1 x Reagent Diluent to a final volume of 50 µL and vortex briefly. Make 1:200 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 50 µL of Detection Agent to 10 mL Reagent Diluent prior to the assay. The rest of undiluted Detection Reagent can be stored at 4°C for up to 6 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
20 x Wash Buffer, 25 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent, 3 mL – stored at -20°C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
Substrate Solution, 10 mL.
Stop Solution, 5 mL.

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Assay Procedure

1. Lift the plate cover from the top left corner and cover the wells that are not used. Vortex briefly the samples and the standards prior to the assay. Add 100 μL of sample (such as plasma or serum) or standard to each well, cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash Buffer (300 μL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 μL of the working dilution of the Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 μL of the working dilution of Detection Agent to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 μL of Substrate Solution to each well. Incubate for 5-10 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, 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.

Precaution and Technical Notes

1. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standard at each of dilution step is critical to ensure a normal standard curve.
2. If VEGF exceeds the upper limit of the detection, the sample needs to be diluted with the Sample Diluent. The dilution factor must be used for calculation of the concentration.
3. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
4. The Stop Solution is an acid solution, handle with caution.
5. This kit should not be used beyond the expiration date on the label.
6. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
7. Use a fresh reagent reservoir and pipette tips for each step.
8. It is recommended that all standards and samples be assayed in duplicate.
9. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

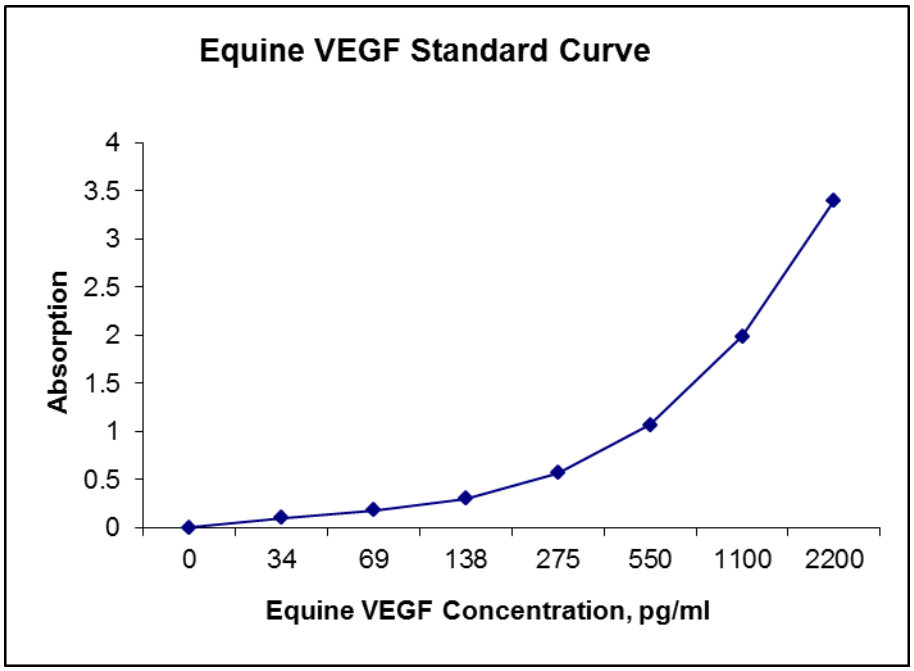
Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero (blank) standard optical density.

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 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 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.

The Standard Curve

The graph below represents typical data generated when using this Equine VEGF ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark^{TM} Microplate Reader and a Microplate Manager 6 Software were used to generate this curve. The correlation coefficient (r^2) is 0.999-1.000.



Specificity

The following recombinant Equine proteins prepared at 1 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP1, BMP2, BMP4, HGF, IL-1β, IL-1RA, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFNγ, MMP-2, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α.

Calibration

This kit is calibrated against a highly purified CHO cell-expressed recombinant equine VEGF.

Detection Range

1-2200 pg/ml

Assay Sensitivity

0.5 pg/ml

Assay Precision

Intra-Assay %CV: 6; Inter-Assay %CV: 9

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Related products

20 x Sample Diluent, GERC-103058
20 x PBS, Cat. 103004-20
10 x ELISA Wash Buffer, Cat. 103028
10 x ELISA Reagent Diluent, Cat. GERC-103055
Universal Blocking Buffer, Cat.103005
2 x Recombinant Protein Stabilizer, Cat. GERC-03014-2
5 x Recombinant Protein Stabilizer, Cat. GERC-103014-5
ELISA G-Blue Substrate Solution, Cat. 103021
Equine VEGF Standard
Equine VEGF detection antibody