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Research
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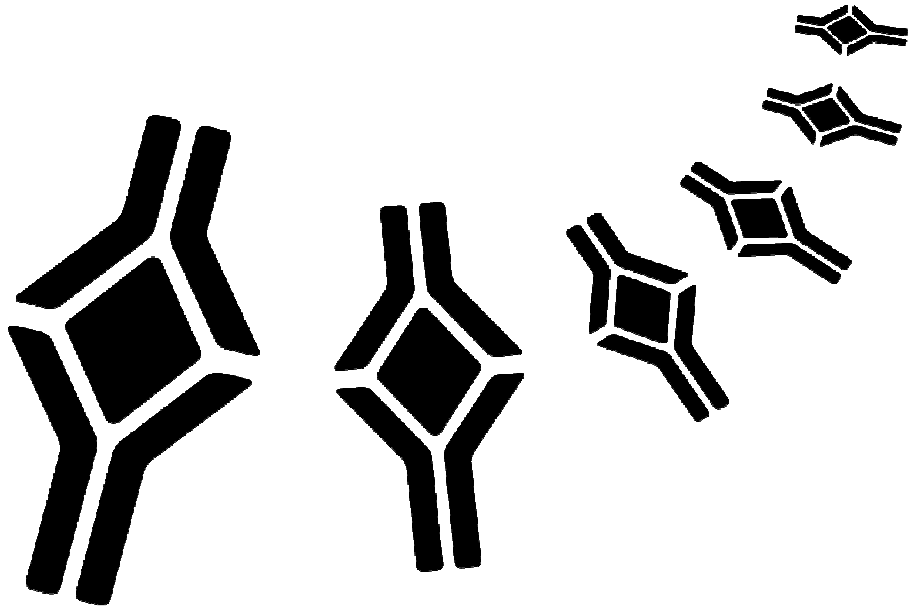


# HUMAN VEGFR1/FLT1 ELISA

Product Data Sheet

Cat. No.: BBT0543R

For Research Use Only



VERSION 51 070510

# CONTENTS



**This kit is manufactured by:**
**BioVendor – Laboratorní medicína a.s.**

**Use only the current version of Product Data Sheet enclosed with the kit!**

## 1. INTENDED USE

For quantitative detection of human VEGFR1 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

## 2. STORAGE, EXPIRATION

**Storage**
Store at 4℃ for frequent use, at -20℃ for infrequent use.
Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

**Expiration**
Four months at 4℃ and eight months at -20℃.

## 3. INTRODUCTION

VEGFR1, also known as FMS-related tyrosine kinase 1(FLT1). Oncogene FLT belongs to the src gene family and is related to oncogene ROS . Like other members of this family, it shows tyrosine protein kinase activity that is important for the control of cell proliferation and differentiation. FLT is mapped to 13q12. VEGF receptor 1 signaling is essential for osteoclast development and bone marrow formation in colony-stimulating factor 1-deficient mice. The standard product used in this kit is recombinant human VEGFR1, consisting of 905 amino acids with the molecular mass of 100KDa

## 4. TEST PRINCIPLE

Human VEGFR1 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human VEGFR1 specific-specific monoclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human VEGFR1 amount of sample captured in plate.

## 5. PRECAUTIONS

- Before using Kit, spin tubes and bring down all components to bottom of tube.
- Duplicate well assay was recommended for both standard and sample testing.
- Don't let 96-well plate dry, dry plate will inactivate active components on plate.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37℃ for 30 min before using.

## 6. REAGENT SUPPLIED

- Lyophilized recombinant human VEGFR1 standard: 10 ng/tube×2.
- One 96-well plate precoated with anti- human VEGFR1 antibody.
- Sample diluent buffer: 30 ml
- Biotinylated anti- human VEGFR1 antibody: 130 µl, dilution 1:100.
- Antibody diluent buffer: 12 ml.
- Avidin-Biotin-Peroxidase Complex (ABC): 130 µl, dilution 1:100.
- ABC diluent buffer: 12 ml.
- TMB color developing agent: 10 ml.
- TMB stop solution: 10 ml.

## 7. MATERIAL REQUIRED BUT NOT SUPPLIED

- Microplate reader in standard size.
- Automated plate washer.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- Clean tubes and Eppendorf tubes.
- Washing buffer (neutral PBS or TBS).
  Preparation of 0.01M **TBS:** Add 1.2g Tris, 8.5 g Nacl; 450 µl of purified acetic acid or 700 µl of concentrated hydrochloric acid to 1000 ml $H_2O$ and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
  Preparation of 0.01 M **PBS:** Add 8.5 g sodium chloride, 1.4 g $Na_2HPO_4$ and 0.2g $NaH_2PO_4$ to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

## 8. PREPARATION OF REAGENTS AND SAMPLES

**Plate Washing**

Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes.

Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.

**Sample Preparation and Storage**

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C.

Avoid repeated freeze-thaw cycles.

Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C

Serum: Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

Plasma: Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 min at 2-8℃ at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store samples at -20°C.

**Sample Dilution Guideline**

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer.

The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

**High target protein concentration (100-1000 ng/ml)**. The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.

**Medium target protein concentration (10-100 ng/ml)**. The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.

**Low target protein concentration (156-10,000 pg/ml)**. The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.

**Very Low target protein concentration (≤156 pg/ml)**. No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

A. Reconstitution of the human VEGFR1 standard: VEGFR1 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of VEGFR1 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.
   a. 10,000 pg/ml of human VEGFR1 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
   b. 5000 pg/ml→156 pg/ml of human VEGFR1 standard solutions: Label 6 Eppendorf tubes with 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312 pg/ml, 156 pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10,000 pg/ml VEGFR1 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.
      Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.
B. Preparation of biotinylated anti-human VEGFR1 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
   a. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
   b. Biotinylated anti-human VEGFR1 antibody should be diluted in 1:99 with the antibody diluent buffer and mixed thoroughly.
C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
   a. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
   b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:99 with the ABC dilution buffer and mixed thoroughly.

# 9. ASSAY PROCEDURE

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard VEGFR1 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of VEGFR1 amount in samples.

1. Aliquot 0.1 ml per well of the 10,000 pg/ml, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312 pg/ml, 156 pg/ml human VEGFR1 standard solutions into the precoated 96-well plate. Add 0.1 ml of the sample diluent buffer into the control well (Zero well). Add 0.1 ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each human VEGFR1 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 ml of biotinylated anti-human VEGFR1 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
6. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C for 25-30 min (shades of blue can be seen in the wells with the four most concentrated human VEGFR1 standard solutions; the other wells show no obvious color).
9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human VEGFR1 concentration of the samples can be interpolated from the standard curve.

**Note:** if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

**Summary**

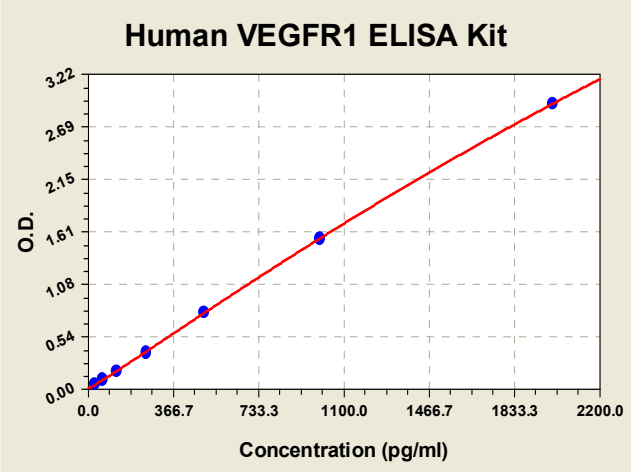
1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C for 25-30 min.
5. Add TMB stop solution and read.

## 10. CALCULATIONS

**Typical Data Obtained from Human VEGFR1**
(TMB reaction incubate at 37°C for 30 min)

| Concentration | 0.0 pg/ml | 31.2 pg/ml | 62.5 pg/ml | 125 pg/ml | 250 pg/ml | 500 pg/ml | 1000 pg/ml | 2000 pg/ml |
|---|---|---|---|---|---|---|---|---|
| O.D. | 0.009 | 0.062 | 0.117 | 0.186 | 0.390 | 0.796 | 1.554 | 2.931 |

**Typical Human VEGFR1 ELISA Kit Standard Curve**
This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.



## 11. PERFORMANCE CHARACTERISTICS

**» Typical analytical data of BioVendor Human VEGFR1/FLT1 ELISA are presented in this chapter.**

- **Sensitivity**

< 4 pg/ml

- **Specificity**

No detectable cross-reactivity with any other cytokine.

- **Range**

156 pg/ml-10, 000 pg/ml

- **Size**

96T

## 12. REFERENCES

**References to human VEGFR1/FLT1 ELISA:**


1. 1 Imbert, A.; Rosnet, O.; Marchetto, S.; Ollendorff, V.; Birnbaum, D.; Pebusque, M.-J. : Characterization of a yeast artificial chromosome from human chromosome band 13q12 containing the FLT1 and FLT3 receptor-type tyrosine kinase genes. Cytogenet. Cell Genet. 67: 175-177, 1994.
2. Niida, S.; Kondo, T.; Hiratsuka, S.; Hayashi, S.-I.; Amizuka, N.; Noda, T.; Ikeda, K.; Shibuya, M. : VEGF receptor 1 signaling is essential for osteoclast development and bone marrow formation in colony-stimulating factor 1-deficient mice. Proc. Nat. Acad. Sci. 102: 14016-14021, 2005.


**» For more references on this product see our WebPages at www.biovendor.com**

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## NOTES

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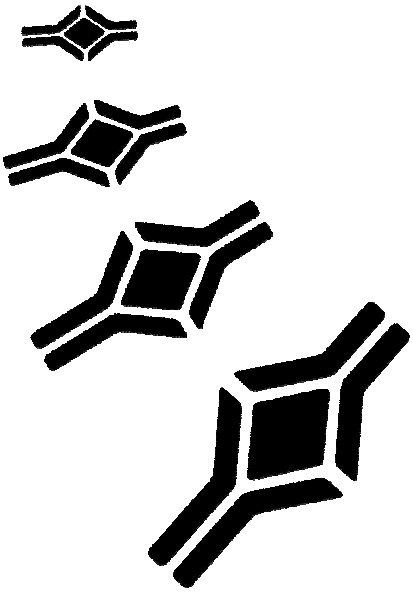


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