ELISA kits available from ADI (see details at the web site)

200-800-AVG **Avastin/Bevacizumab (Anti-VEGF) ELISA Kit for human, 96 tests**
200-810-ADG **Human Anti-Avastin/Bevacizumab IgG (anti-drug IgG) ELISA Kit for human, 96 tests**
200-820-VEF **Human VEGF ELISA Kit, 96 tests**
200-830-VEM **Mouse VEGF ELISA Kit, 96 tests**
200-840-VER **Rat VEGF ELISA Kit, 96 tests**
200-850-FLT **Human VEGFR1/FLT1 ELISA Kit, 96 tests**
200-860-KDR **Human VEGFR2/KDR ELISA Kit, 96 tests**
200-870-ID24 **Avastin/Bevacizumab identification/Counterfeit detection ELISA Kit, 24 tests**

#0010 Human Leptin
#200-120-AGH Human globular Adiponectin (gAcrp30)
#0700 Human Sex Hormone Binding Glob (SHBG)
#0900 Human IGF-Binding Protein 1 (IGFBP1)
#1000 Human C-Reactive Protein (CRP)
#100-110-RSH Human Resistin /FIZZ3
#100-140-ADH Human Adiponectin (Acrp30)
#100-160-ANH Human Angiogenin
#100-180-APH Human Angiopoietin-2 (Ang-2)
#100-190-B7H Human Bone Morphogenic Protein 7 (BMP-7)
#1190 Human Serum Albumin **#1200** Human Albumin (Urinary)
#1750 Human IgG (total) **#1760** Human IgM
#1800 Human IgE **#1810** Human Ferritin
#1210 Human Transferrin (Tf) **#0020** Beta-2 microglobulin
#1600 Human Growth Hormone (GH)

#0060 Human Pancreatic Colorectal cancer (CA-242)
#1820 Human Ovarian Cancer (CA125) **#1830** Human CA153
#1840 Human Pancreatic & GI Cancer (CA199)
#1310 Human Pancreatic Lipase
#1400 Human Prostatic Acid Phosphatase (PAP)
#1500 Human Prostate Specific Antigen (PSA) **#1510** free PSA (fPSA)
#0500 Human Alpha Fetoprotein (AFP)
#0050 Human Neuron Specific Enolase (NSE)

#0030 Human Insulin **#0040** Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle Stimulating Hormone (FSH)
#0300 Human Prolactin (PRL)
#0400 Human Chorionic Gonadotropin (HCG) #**0410** HCG-free beta

#0600 Human Thyroid Stimulating Hormone (TSH)
#1100 Human Total Thyroxine (T4) **#1110** Human Free T4 (fT4)
#1650 Human free triiodothyronine (fT3) **#1700** Human T3 (total)

#3000 Human Rheumatoid Factors IgM (RF)
#3100 Human anti-dsDNA

Instruction Manual No. M-200-850-FLT

Human soluble fms like tyrosine kinase (sFlt-1)

ELISA KIT # 200-850-FLT, 96 Tests

For Quantitative measurement of soluble form of VEGF receptor known as sVEGFR1 /sFlt-1 in human serum, plasma and culture supernatant



For In Vitro Research Use Only (RUO)

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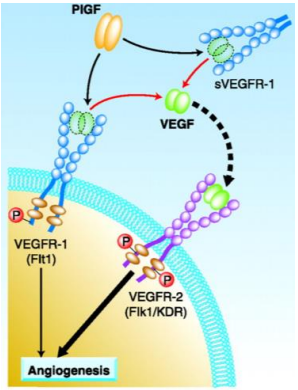
Human sVEGFR1/sFLT1 ELISA KIT # 200-850-FLT, 96 Tests

Kit Components	Cat #
Anti-hVEGFR1/FLT1 IgG coated strips, 96 wells, #200851	1 plate
Human FLT1, Recombinant, **lyophilized standard** (10 ng), 2 vials; make more standards #200852	2 vials
Biotinylated Anti-hVEGFR1 antibody (100X), 100 µl, #200853	1 vial
Avidin-Biotin-Peroxidase complex (ABC) (**100X**), 100 µl, #200854	1 vial
Sample diluent buffer, 30 ml, # 200855	1 bottle
Antibody Diluent buffer, 12 ml, # 200856	1 bottle
ABC Diluent buffer, 12 ml, # 200857	1 bottle
TMB Substrate Solution, 10 ml, # 200850-TMB	1 bottle
Wash buffer (25X), 20 ml (dilute 1:25 with distilled water, **20 ml stock to 480 ml dH2O**) , # 200850-WB	1 bottle
Stop solution, 10 ml, # 200850-SS	1 bottle
Complete Instruction Manual, M-200850-FLT	1

Intended use

Human sVEGFR1/sFLT1 ELISA kits is for Quantitative measurement of soluble form of VEGF receptor known as sVEGFR1/sFlt-1 in human serum, plasma and culture supernatant. **For research use only (RUO),** not for diagnosis, cure or prevention of the disease.

Introduction



Vascular endothelial growth factor A (**VEGF/VEGF-A**), a dimeric glycoyslated protein, is a member of the platelet-derived growth factor (PDGF/VEGF) family. VEGFA is essential for adults during organ remodeling and diseases that involve blood vessels, for example, in wound healing, tumor angiogenesis, diabetic retinopathy, and age-related muscular degeneration. VEGF-A is produced as a group of three major isoforms as a result of alternative splicing and if any three isoforms are produced (VEGF-A120, VEGF-A164, and VEGF-A188). VEGF-A could be used to treat patients with neurodegenerative and neuropathic conditions. VEGF-A mediates the growth of new blood vessels from pre-existing vessels (angiogenesis) by binding to the cell surface receptors **VEGFR1 (FLT1)** and **VEGFR2 (KDR)**. These two receptors act through different pathways to contribute to endothelial cell proliferation and migration, and formation of tubular structures.

Anti-VEGFA therapy can be used to treat patients with undesirable angiogenesis and vascular leakage in cancer and eye diseases but also could result in the inhibition of neurogenesis and neuro-protection. Bevacizumab (trade name Avastin, Genentech/Roche) is a humanized monoclonal antibody that inhibits vascular endothelial growth factor A (VEGF-A). Bevacizumab binds directly to VEGF to form a protein complex which is incapable of further binding to VEGF receptor sites (which would initiate vessel growth) effectively reducing available VEGF. The Bevacizumab/VEGF complex is both metabolized and excreted directly. Many diseases of the eye, such as age-related macular degeneration (AMD) and diabetic retinopathy, damage the retina and cause blindness when blood vessels around the retina grow abnormally and leak fluid, causing the layers of the retina to separate.

Performance Characteristics

Range

156 pg/ml-10000pg/ml(human serum, plasma)

156 pg/ml-10000pg/ml(cell culture supernates)

Sensitivity

< 30 pg/ml

Specificity

Natural and recombinant human sVEGFR1

Cross-reactivity

No detectable cross-reactivity with other relevant proteins

Species cross-reactivity

The antibodies (anti-human VEGF) used this kit has not been tested for potential cross-reactive with VEGF from species like monkey, mouse, and rat etc. ADI has separate ELISA kits for mouse and rat VEGF.

Quick Summary of VEGFR1/sFLT1 ELISA

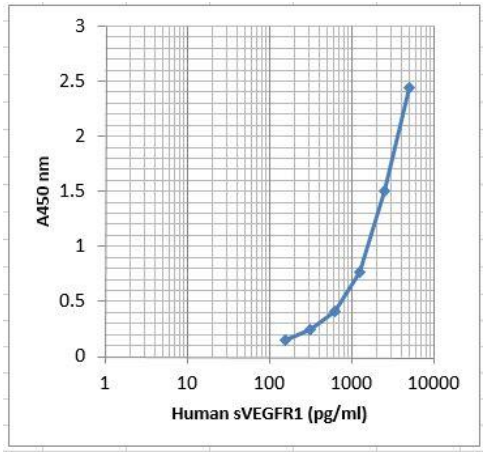
1. Add **0.1 ml samples and standards and incubate the plate at 37°C for 90 min**. Remove the well content by aspiration or flicking over the waster container then blotting the wells over paper towels. Do not wash.
2. **Add 0.1 ml 1X biotinylated antibodies** and **incubate the plate at 37°C for 60 min**.
3. **Wash plate 3 times** with wash buffer (300 ul/wash).
4. **Add 0.1 1X ABC solution** and **incubate the plate at 37°C for 30 min**.
5. **Wash plate 5 times** with wash buffer
6. Add **90 ul TMB and incubate the plate at 37°C in dark for 20-25 min**.
7. Add **100 TMB stop solution** and **read at 450nm/630nm**.

References: Maglione D (1991) PNAS 88, 9267-9271; Autiero M (2003) Nature Med. 9, 936-943; Luttun A (2002) Nature Med. 8, 831-840;

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	A450 Net Abs.
A1, A2	**blank** (0 pg/ml)	0.059
B1, B2	**Std. A** (15.6 pg/ml)	0.104
C1, C2	**Std. B** (31.2 pg/ml)	0.151
D1, D2	**Std. C** (62.5 pg/ml)	0.243
E1, E2	**Std. D** (125.0 pg/ml)	0.412
F1, F2	**Std. E** (250.0 pg/ml)	0.771
G1, G2	**Std. F** (500.0 pg/ml)	1.503
H1, H2	**Std. G** (1000.0 pg/ml)	2.442

NOTE: These data are for demonstration purpose only. A complete std. curve must be run in every assay to determine sample values.



*Ar/5_ADI_ELISA-graph

A typical std curve (Plot linear graph Draw the best curve through the points. Do not use this for calculating sample values).

Calculations

Subtract the A450 values of blank or zero wells from all values. Plot A450 values of the standard on (Y) axis vs. concentration (X) on a semi-log scale. The sample VEGF concentration 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.

PRINCIPLE OF THE TEST

Human VEGF ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for VEGF has been precoated onto 96-well plates. Standards and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for VEGF is added 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 VEGF amount of sample captured in plate.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The VEGF ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions.
Applicable **MSDS**, if not already on file, **for** the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

1. 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 supernates**: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) 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 heparin, EDTA or citrate as an anticoagulant. Centrifuge for 20 min at 2000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

2. 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 (100-1000ng/ml). The working dilution is 1:100. i.e. Add 1µl sample into 99 µl sample diluent buffer.
- **Medium target protein** concentration (10-100ng/ml). The working dilution is 1:10. i.e. Add 10µl sample into 90 µl sample diluent buffer.
- **Low target protein** concentration (156-10000pg/ml). The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein** concentration (≤31.2pg/ml). No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

1. Reconstitution of the human VEGFR1 standards:

VEGF/FLT1 stds are provided as lyophilized vials (2 at 10 ng/tube). VEGF standard solution should be prepared no more than 2 hours prior to the experiment. Use one tube for each experiment. **Note:** The standard solutions are best used within 2 hours. The 10ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

We recommend the following scheme to prepare **Stds A-G** that will be used for the stand. curve. Add 1 ml sample diluent buffer into one tube, mix gently and leave the tube at room temp. for 10 min and. Stock standard of 10,000 pg/ml of human VEGF(std H): Do not vortex vigorously. Need 200 ul of each standard per run (100 ul/well in duplicate).

Stds	Sample diluent	Total volume	**Final Conc pg/ml**
0.1 ml of Std H (10,000 pg/ml)	0.4 ml	0.5 ml	**Std G**, 2000 pg/ml
Stds G-A are prepared by 2-fold serial dilutions			
Std G, 2000 pg/ml (0.25 ml)	0.25 ml	0.5 ml	**Std F**, 1000 pg/ml
Std F, 1000 pg/ml (0.25 ml)	0.25 ml	0.5 ml	**Std E**, 500 pg/ml
Std E 500 pg/ml (0.25 ml)	0.25 ml	0.5 ml	**Std D**, 250 pg/ml
Std D, 250 pg/ml (0.25 ml)	0.25 ml	0.5 ml	**Std C**, 125 pg/ml
Std C, 125 pg/ml (0.25 ml)	0.25 ml	0.5 ml	**Std B**, 62.5 pg/ml
Std B, 62.5 pg/ml (0.25 ml)	0.25 ml	0.5 ml	**Std A**, 31.2 pg/ml

Note: Gently mix the standard and diluent with the pipette before taking the standard for the next dilution.

2. Preparation of 1x-biotin anti-human VEGF antibody solution:

The solution should be prepared no more than **2 hours** prior to the experiment.

Biotinylated anti-human VEGF antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 10 µl Biotinylated anti-human VEGF antibody to 990 µl antibody diluent buffer or 0.ml stock in 9.9 ml diluent). Prepare 1 ml for each strip or 10 ml for the full plate. Need 100 ul/well.

3. Preparation of 1X-Avidin-Biotin-Peroxidase Complex (ABC) solution:

Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in **1:100 with the ABC dilution buffer** and mixed thoroughly. (i.e. Add 10 µl ABC to 990 µl ABC diluent buffer or 100 ul to 9.9 ml diluent.)
The solution should be prepared no more than 1 hour prior to the experiment. Need 0.1ml/well. Prepare 1 ml for 1 strip or 10 ml for full plate.

4. Prepare wash buffer (25X): Dilute (1:25) with distilled water (20 ml stock to final volume of 500 ml i.e.; (**20 ml conc with 480 ml distilled water**). Store at 4°C.

STORAGE AND STABILITY
The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Once opened/used standards are stable for two month at 2-8^{o} C.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

TEST PROCEDURE

Prepare standards, samples, and all reagents before using the kit. 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. Std. VEGF detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of VEGF amount in samples. Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. **Add 0.1 ml per well of the standards, samples or controls** (Stds A-G prepared on page 3) into appropriate wells. **Add 0.1 ml of the sample diluent buffer** into the control well (Zero well). We recommend that each human VEGF 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. No washing is necessary.

4. **Add 0.1ml of 1x biotinylated anti-human FLT1 antibody** working solution into each well and incubate the plate at **37°C for 60 min**.

5. **Wash plate 3 times** with 1x wash buffer (300 ul/well), 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. Repeat this process two additional times for a total of THREE washes. **Note**: For automated washing, aspirate all wells and wash THREE times with 300 ul wash buffer. Blot the plate onto paper towels or other absorbent material.)

6. **Add 0.1ml of prepared 1X ABC working solution** into each well and **incubate the plate at 37°C for 30 min**.

7. **Wash plate 5 times** as in step 5.

8. **Add 90 µl of prepared TMB color** developing agent into each well and incubate plate at **37°C in dark for 20-25 min** (Note: blue color develops in 4 highest standards and positive samples. **T**he other wells show no obvious or intense color). Note: TMB incubation time may be varied 5-10 mins so as to get the highest standards A450 reading of 2.00-3.00 or within the linear reading range of most ELISA readers.

9. **Add 0.1 ml of prepared TMB stop solution into each well**. The color changes into yellow immediately. :

10. **Read the plates 450nm/630nm** (reference filter) in an ELISA reader within 30 min after adding the stop solution.