

Humanized Anti-VEGF(Avastin/Bevacizumab/Lucentis) ELISA Kits

Vascular endothelial growth factor A (**VEGF/VEGF-A**), a dimeric glycosylated protein, is a member of the platelet-derived growth factor (PDGF/VEGF) family. In vitro, VEGF-A has been shown to stimulate endothelial cell mitogenesis and cell migration. VEGF-A is also a vasodilator and increases microvascular permeability and was originally referred to as vascular permeability factor. 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. During early vertebrate development, vasculogenesis occurs which means that the endothelial condense into the blood vessels. 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 and also increase vascular permeability which will stop the blood-brain barrier and increase inflammatory cell infiltration. 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)**, two tyrosine kinases located in endothelial cells of the cardiovascular system. These two receptors act through different pathways to contribute to endothelial cell proliferation and migration, and formation of tubular structures.

Ranibizumab/Lucentis(Genentech), a Fab fragment derived from the same parent molecule as bevacizumab, has been approved for intracocular use. Bevacizumab is usually given intravenously every 14 days. In colon cancer, it is given in combination with the chemotherapy drug 5-FU (5-fluorouracil), leucovorin, and oxaliplatin or irinotecan. Ranibizumab, like bevacizumab, binds all active isoforms of VEGF-A and is thus considered a non-selective VEGF-A inhibitor.

Avastin, manufactured by Genentech unit of Roche, generated global sales of about \$4.3 billion. Bevacizumab is one of the most expensive drugs widely marketed. It cost about 50,000-100,00 in the USA to treat one patient. Due to high cost, it is an attractive target of adulteration and counterfeit.



Counterfeit Avastin has been distributed in the United States. It contained salt, starch, citrate, isopropanol, propanediol, t-butanol, benzoic acid, di-fluorinated benzene ring, acetone and phthalate moiety, but no active ingredients of the cancer drug. All cartons and vials of authentic Avastin have "Genentech" or "Genentech, a member of the Roche Group" printed on the labels.

But the counterfeit packaging or vials are labeled with Roche as the manufacturer. In a related action, FDA has issued letters to 19 medical practices in the United States that purchased unapproved cancer medicines, including counterfeit Avastin, from Quality Specialty Products, a foreign supplier that may also be known as Montana Health Care Solutions. Volunteer Distribution in Gainesboro, Tennessee is a distributor of Quality Specialty's products. **More than 10% of medicines worldwide, and > 25% of medicines sold in developing countries, are counterfeit according to the World Intellectual Property Organization.** The Center for Medicine in the Public Interest reports that worldwide counterfeit sales are increasing at about 13 percent annually – nearly twice the pace of legitimate pharmaceuticals – and are a \$75 billion industry. Therefore, there is an urgent need to develop rapid tests that can be used by the doctors, pharmacies, and the law enforcement agencies to identify the drug, confirm its concentration and bioactivity.

Like many humanized antibodies, Avastin can induce antibodies (**human anti-Avastin or anti-drug antibodies, HABA/ADA**) in patients receiving Avastin. However, this is highly dependent upon the sensitivity **of the Assay**. In Patients developed with Avastin ADA that also developed ELISA kits to detect antibodies to Avastin (**Human Anti-Avastin Antibodies**) in patients receiving long-term treatments. Additional ELISA kits are also available to measure **VEGF, VEGFR1, and VEGFR2** levels. In addition, ADI is also making simple and easy ELISA kits to detect counterfeit Avastin or adulteration.



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 neuroprotection. **Bevacizumab** (trade name **Avastin**, Genentech/Roche) is a humanized monoclonal antibody that inhibits vascular endothelial growth factor A (VEGF-A). Bevacizumab was approved by the U.S. Food and Drug Administration (FDA) for certain metastatic cancers, lung cancers, renal cancers, and glioblastoma multiforme of the brain. Avastin was the first commercially available angiogenesis inhibitor. 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.

Avastin/Bevacizumab ELISA Kit and related items

| Catalog# | Product Description |
|---------------------|---|
| 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 |
| 200-830-VER | Rat VEGF ELISA Kit, 96 tests |
| 200-850-FLT | Human VEGFR1/FLT1 ELISA Kit for human samples, 96 tests |
| 200-860-KDR | Human VEGFR2/KDR ELISA Kit for human samples, 96 tests |
| 200-870-ID24 | Avastin/Bevacizumab identification/Counterfeit detection ELISA Kit, 24 tests |

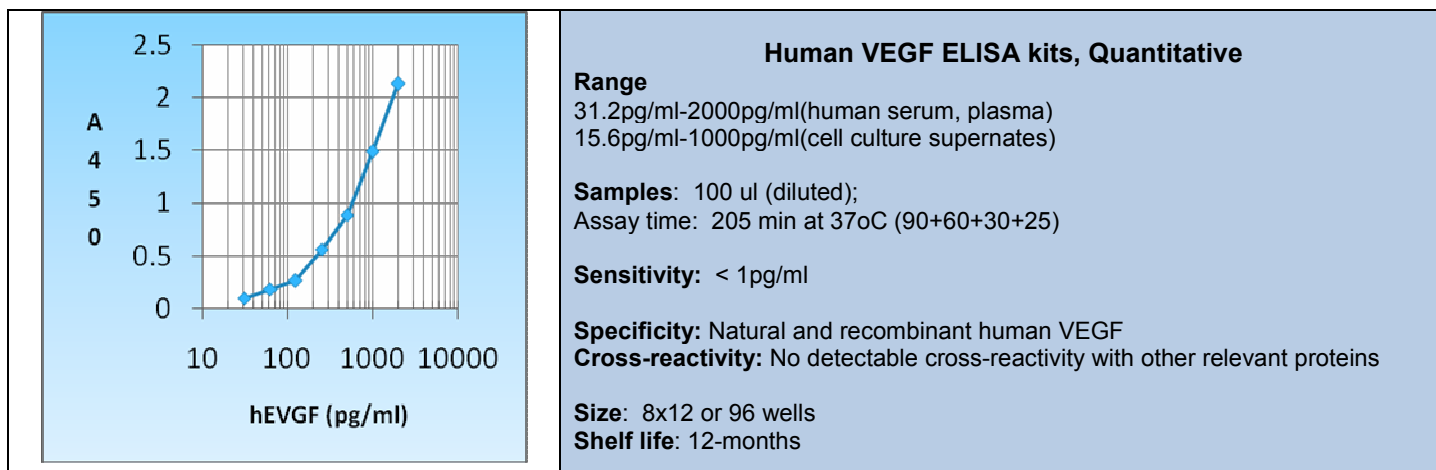
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Human Vascular endothelial growth factor (VEGF) ELISA Kit, 96 tests cat# 200-820-VEF



Brief assay procedure for VEGF ELISA

1. Pipet **100 ul each of diluted standards (0-2000 pg/ml)**, 100 ul samples (diluted as per sample concentrations)
2. Mix gently for 5-10 seconds and incubate for **60 min at 37oC**.
3. Remove the well contents and add **Add 100 ul of detection Antibody**. Mix gently and incubate at 37oC for **60 min**.
4. **Wash 3X using wash buffer**. Add **100 ul of detection antibody-HRP Conjugate**. Mix gently and incubate at 37oC for **30 min**.
5. **Wash 5X using wash buffer**. Add **100 ul of TMB substrate**. Mix gently, cover the plate and incubate at 37oC for **20-30 min**. Blue color develops in standards and positive samples.
6. Pipet **100 ul of stop solution** into all tubes, mix gently (blue color turns yellow). **Measure OD at A450 nm**. Sample concentrations are calculated from the std. curve.

List of Avastin/VEGF Related reagents and ELISAs available from ADI.

Product details, data sheets, and pricing available (http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2785)

| AntibodyType | Catalog# | Product Description |
|--------------|-------------|---|
| | AP-334-U | VEGF receptor Flt-1 (F56), Peptide Aptamer, Unlabeled |
| | AP-335-U | VEGF receptor KDR and Flt-1 (v107), Peptide Aptamer, Unlabeled |
| | AP-336-U | VEGF receptor KDR/Flk-1 (K237), Peptide Aptamer, Unlabeled |
| | AP-337-U | VEGF-KDR, Peptide Aptamer, Unlabeled |
| Rabbit-Poly | FLK11-A | Anti-Mouse FLK-1/VEGFR-2 IgG #1, aff pure |
| Mono-Mice | FLK13-MB | Biotinylated-Mouse Monoclonal Anti-Human FLK-1/VEGFR-2 IgG # 3 |
| Rabbit-Poly | FLT11-A | Anti-human FLT-1/VEGFR-1 IgG #1, aff pure |
| Mouse-Mono | FLT12-M | Mouse Monoclonal Anti-human FLT-1/VEGFR-1 IgG, aff pure |
| Rabbit-Poly | FLT41-A | Anti-Mouse FLT-4/VEGFR-3 IgG #1, aff pure |
| Rabbit-Poly | FLT42-A | Anti-Human FLT-4/VEGFR-3 IgG #2, aff pure |
| Mono-mice | FLT43-M | Mouse monoclonal Anti-Human FLT-4/VEGFR-3 IgG #1, aff pure |
| Mono-Rat | FLT44-M | Rat monoclonal Anti-Mouse FLT-4/VEGFR-3 IgG #1, aff pure |
| Mono-Mice | VEGF12-M | Mouse Monoclonal Anti-Human VEGF protein (1-165) protein IgG #2 |
| | VEGF24-R-10 | Human Recombinant VEGF121 Protein (Sf9), biologically active |
| | VEGF27-R-10 | Human Recombinant VEGF165 Protein (Sf9), biologically active |
| | VEGF34-R-10 | Mouse Recombinant VEGF165 (VEGF-A) Protein (Sf9), biologically active |
| | VEGF35-R-10 | Mouse Recombinant VEGF165 (VEGF-A) Protein (E. coli), biologically active |
| | VEGF36-R-5 | Human Recombinant EG-VEGF Protein (84aa, E. coli), biologically active |

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