

**User's Manual**

# Human EG-VEGF ELISA Development Kit

**REF** DEIA096

15 plates, 45 plates

**RUO**

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

---

**Creative Diagnostics** **Address:** 45-1 Ramsey Road, Shirley, NY 11967, USA **Tel:** 1-631-624-4882 (USA) 44-161-818-6441 (Europe)  **Fax:** 1-631-938-8221 **Email:** [info@creative-diagnostics.com](mailto:info@creative-diagnostics.com)  **Web:** [www.creative-diagnostics.com](http://www.creative-diagnostics.com)

## PRODUCT INFORMATION

### Intended Use

Human EG-VEGF ELISA Development Kit contains the key components required for the quantitative measurement of natural and/or recombinant EG-VEGF in a sandwich ELISA format. Using the ELISA protocol described below, this kit provides sufficient reagents to assay EG-VEGF in approximately 1500 ELISA plate wells.

### General Description

Endocrine Gland-Derived Vascular Endothelial Growth Factor (EG-VEGF) or Prokineticin-1 (PK-1) is a novel secreted protein expressed in various tissues including steroidogenic glands. Although structurally non-related to VEGF, EG-VEGF/PK-1 was shown to promote angiogenesis acting via two G-protein coupled receptors (PK-R1 and PK-R2).<sup>1</sup> EG-VEGF is mitogen specific for the endothelium of steroidogenic glands. It induces proliferation, migration and fenestration in capillary endothelial cells derived from endocrine glands. EG-VEGF mRNA expression is localized to the steroid hormone-producing cells, i.e. Leydig cells of the testis and the specialized stroma in the ovary.<sup>2</sup> EG-VEGF increased the phosphorylation of Akt in a phosphatidylinositol 3-kinase-dependent manner and is a survival factor for ACE cells. EG-VEGF, possibly through binding to a G-protein coupled receptor, results in the activation of MAPK p44/42 and phosphatidylinositol 3-kinase signaling pathways, leading to proliferation, migration, and survival of responsive endothelial cells.

### Reagents And Materials Provided

1. Capture Antibody
2. Detection Antibody
3. Standard
4. StreptAvidin-HRP
5. TMB Liquid Substrate "Ready to Use"

### Materials Required But Not Supplied

#### Additional Required Materials

ELISA microplates

BSA

Dulbecco's PBS (DPBS) [10x]

Stop Solution: 450 nm Stop Reagent for TMB Microwell

#### Required Solutions

PBS: dilute 10xPBS to 1xPBS, pH 7.2, in sterile water

Wash Buffer: 0.05% Tween-20 in PBS

Reagent Diluent: 1.0% BSA in PBS\*

Blocking Buffer 1.0% BSA in PBS

Note: Other acceptable blocking buffers such as CD's Ultra-FISH Block or Ultra-Synthetic Block may be used for assay optimization.

## Storage

See "Reagent Preparation"

## Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS without carrier protein and immediately add 100 µL to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash the plate 4 times using 300-400 µL of wash buffer per well.

Note: We recommend using an autowasher, although a squirt bottle or manifold dispenser would suffice.

3. After the last wash, invert plate to remove residual buffer and blot on paper towel.
4. Add 300 µL block buffer to each well and incubate for at least 1 hour at room temperature.
5. Aspirate and wash plate 4 times.

Note: Complete removal of liquid at each step is essential for good performance and sensitivity of assay.

## Reagent Preparation

Mouse Anti-Human EG-VEGF Capture Antibody: Centrifuge vial prior to opening. Reconstitute in 1.0 mL sterile PBS. Refer to the lot-specific datasheet for amount supplied and dilute in PBS without carrier protein to the working concentration indicated on the C of A. Following reconstitution the capture antibody may be stored at 2-8°C for up to 6 months. For long term storage, it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer. Avoid repeated freeze and thaw cycles.

Biotinylated Goat Anti-Human EG-VEGF Detection Antibody: Refer to the lot-specific datasheet for amount supplied. Centrifuge vial prior to opening. Reconstitute with 1.0 mL of Reagent Diluent. Dilute in Reagent Diluent to the working concentration indicated on the C of A. Detection antibodies may be stored at 2-8°C for up to 6 months. For long term storage, it is recommended to aliquot into working volumes and store at -70°C in a manual defrost the freezer. Avoid repeated freeze and thaw cycles.

NS0- derived Recombinant Human EG-VEGF Standard: Centrifuge vial prior to opening. Reconstitute each vial with 0.5 mL of Reagent Diluent. Refer to the lot-specific datasheet for amount supplied. The rProtein may be stored at 2-8°C for one (1) month or aliquoted and stored at -70°C for up to three months in a manual defrost freezer. Avoid repeated freeze and thaw cycles.

TMB Liquid Substrate "Ready to Use" (TMB Substrate should be at ambient temperature prior to use): 60.0 mL of TMB HRP Microwell Substrate Standard Kinetic One Component "Ready Use" is provided. The high quality of the substrate can be preserved by storing at temperatures between 2-8°C. When properly stored, TMB Microwell Substrate is stable for a minimum of 48 months from the manufactured date.

## Assay Procedure

**Standard/Sample:** Add 100  $\mu$ L of the working dilution with reagent dilution standard or sample to each well (duplicate recommended). Cover plate with an adhesive plate cover and incubate at room temperature for at least 2 hours.

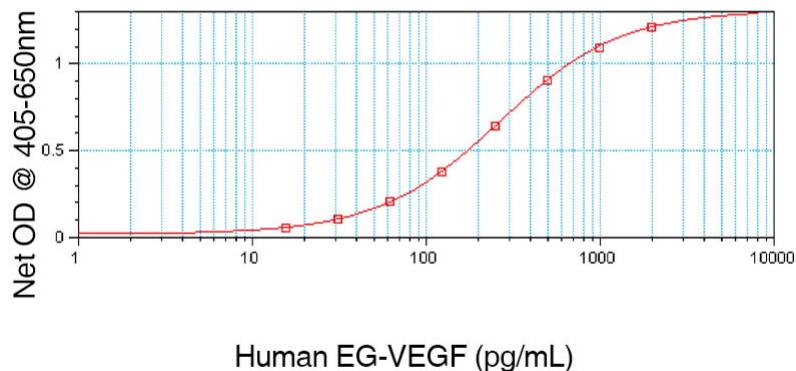
**Detection:** Aspirate and wash plate 4 times. Add 100  $\mu$ L of the detection antibody, diluted in Reagent Diluent to each well. Cover with a new adhesive plate cover and incubate at room temperature for 2 hours.

**StreptAvidin-HRP Conjugate:** Aspirate and wash plate 4 times. Add 100  $\mu$ L of the working dilution (the dilution factor may require optimization) to each well. Cover and incubate at room temperature for 20-30 minutes. Exposure to direct light should be avoided.

**TMB Liquid Substrate:** Aspirate and wash plate 4 times. Add 100  $\mu$ L of TMB HRP Microwell Substrate Standard Kinetic One Component "Ready" "Ready Use" to each well. Incubate at room temperature for 20-30 minutes and monitor color development. Exposure to direct light should be avoided.

**Stop Solution:** Add 50-100  $\mu$ L of Stop Solution to each well. Monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm.

## Typical Standard Curve



## Precautions

Some of the required components may contain acid and/or cause allergic reactions. Breathing in product mist or fumes should be avoided. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

## References

1. Meidan, R. et al. (2005) Endocrinology. 146(9):3950-8.
2. Ferrara, N. et al. (2002) Cold Spring Harb Symp Quant Biol.67:217-21.
3. Ferrara, N. et al. (2002) J Biol Chem. 277(10):8724-9.