



Human Human EG-VEGF ELISA Development Kit

Prod. No.: E148
Lot No.: *Always Refer To Lot Specific Datasheet Provided With Kit for Accurate Instructions*
Pkg. Size: 10 Plates

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).¹ 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.²

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,³ and survival of responsive endothelial cells.³

Human EG-VEGF ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant hEG-VEGF in a sandwich ELISA format within the range of 16–2000pg/ml. Using the ELISA protocol described below, the recommended microplates, reagents and solutions, the components supplied in this kit are sufficient to assay hEG-VEGF in approximately 1000 ELISA plate wells.

Reconstitution & Storage

Capture Antibody: 25µg of antigen-affinity purified goat anti-hEG-VEGF + 2.5mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 0.25ml sterile water for a concentration of 100µg/ml.

Detection Antibody: 25µg of biotinylated antigen-affinity purified goat anti-hEG-VEGF + 2.5mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 0.25ml sterile water for a concentration of 100µg/ml.

Human EG-VEGF Standard: 1µg of recombinant hEG-VEGF + 2.2mg BSA + 11.0mg D-mannitol. Centrifuge vial prior to opening. Reconstitute in 1ml sterile water for a concentration of 1µg/ml.

Note: The reconstituted components are stable for 2 weeks when stored at 2-8°C. Components that have been reconstituted and aliquoted can be stored at -20°C for up to 6 months.

Avidin-HRP Conjugate: 60µl vial. Upon receipt, avidin- HRP conjugate should be aliquoted into ten 6µl vials and stored at <-200C. Aliquots stored frozen at <-200C are stable for up to 2 years from date of receipt. Avoid more than one freeze-thaw cycle.

Recommended Materials

- ELISA microplates (Nunc Maxisorp Prod. #442404);
- Tween-20 (Sigma Cat. # P-7949);
- BSA (Sigma Cat # A-7030);
- TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" (Leinco Prod. T118);
- Dulbecco's PBS [10x] (Gibco BRL Cat. #14200-075).

Recommended Solutions

All solutions should be at ambient temperature prior to use.

PBS: Dilute 10xPBS to 1xPBS, pH 7.20 in sterile water.

Wash Buffer: 0.05% Tween-20 in PBS

Block Buffer: 1% BSA in PBS *



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Diluent: 0.05% Tween-20, 0.1% BSA in PBS *

* Sterile filter and store at 4°C for up to 1 week.

Plate Preparation

1. Dilute capture antibody with PBS to a concentration of 0.25µg/ml. 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 µl of wash buffer per well. After the last wash invert plate to remove residual buffer and blot on paper towel.
3. Add 300µl block buffer to each well. Incubate for at least 1 hour at room temperature.
4. Aspirate and wash plate 4 times.

Elisa Protocol

Standard/Sample: Dilute standard from 2ng/ml to zero in diluent. Immediately add 100µl of standard or sample to each well in triplicate. Incubate at room temperature for at least 2 hours.

Detection: Aspirate and wash plate 4 times. Dilute detection antibody in diluent to a concentration of 0.25µg/ml. Add 100µl per well. Incubate at room temperature for 2 hours.

Avidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute 5.5µl of avidin-HRP conjugate 1:2000 in diluent for a total volume of 11ml. Add 100µl per well. Incubate 30 minutes at room temperature.

ABTS Liquid Substrate:

(ABTS Substrate should be at ambient temperature prior to use)

Aspirate and wash plate 4 times. Add 100µl of substrate solution to each well. Incubate at room temperature for color development. Monitor color development with an ELISA plate reader at 405 nm with wavelength correction set at 650 nm.

NOTE: Reliable standard curves are obtained when either O.D. readings do not exceed 0.2 units for the zero standard concentrations, or 1.2 units for the highest standard concentration. The plate should be monitored at 5-minute intervals for approximately 15 minutes. *O.D. readings may vary.*

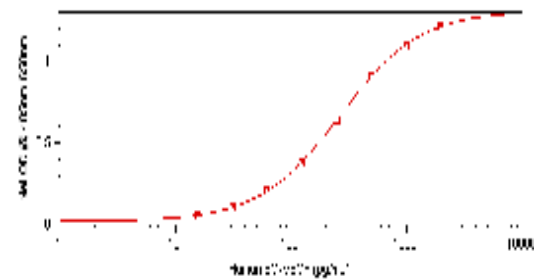
Cross Reactivity

When tested at 50ng/ml the following antigens did not exhibit significant cross reactivity:

Human - EGF, FGF-16, flt3-L, GM-CSF, PDGF-AA, PDGF-AB, PDGF-BB, PIGF, Prokineticin-2, RANTES, SCF, VEGF₁₆₅, VEGF₁₂₁

Murine - EGF, flt3-L, GM-CSF, SCF, VEGF

Rat - EGF, SCF



References

1. Rehovot, Israel *et al.* (2005) *SSR*
2. R Lin & F Peale *et al.* (2003) *Endocrine Abstracts* 5:S11
3. Napoleone Ferrara *et al.* (2002) *J. Biol. Chem* 277:8724



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