

Human PROKE1 ELISA Development Kit

Cat. No. CKE-PROKE1-011R **Lot. No.** (See product label)

SPECIFICATION

Product Overview

Species Human

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). PROKE1 is mitogen specific for the endothelium of steroidogenic glands. It induces proliferation, migration and fenestration in capillary endothelial cells derived from endocrine glands. PROKE1 mRNA expression is localized to the steroid hormone-producing cells, i.e. Leydig cells of the testis and the specialized stroma in the ovary. PROKE1 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. Human PROKE1 ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant hPROKE1 in a sandwich ELISA format within the range of 16–2000 pg/ml. Using the ELISA protocol described below, the recommended microplates, reagents and solutions, the components supplied in this kit are sufficient to assay hPROKE1 in approximately 1000 ELISA plate wells.

RECONSTITUTION & STORAGE

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

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concentration of 100g/ml. Detection Antibody: 25g 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 100g/ml. Human EG-VEGF Standard: 1g 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 1g/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 Prodt. 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 * 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 0.25g/ml. Immediately, add 100μto 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 300μof wash buffer per well. 3. After the last wash invert plate to remove residual buffer and blot on paper towel. 4. Add 300μblock buffer to each well. Incubate for at least 1 hour at room temperature. 5. Aspirate and wash plate 4 times.

ELISA PROTOCOL

Standard/Sample: Dilute standard from 2ng/ml to zero in diluent. Immediately add

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100µof 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 0.25g/ml. Add 100µper well. Incubate at room temperature for 2 hours. Avidin-HRPConjugate: Aspirate and wash plate 4 times. Dilute 5.5µof avidin-HRP conjugate 1:2000 in diluent for a total volume of 11ml. Add 100µper 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 100µof 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, Epigen, Epiregulin, HB-EGF, Heregulinβ-1, Prokineticin-2, TGF-α

GENE INFORMATION**SYNONYMS**

PROK1; prokineticin 1; PK1; PRK1; EGVEGF; EG-VEGF; PK1; Mambakine; OTTHUMP00000013289; PRK1; mambakine; Endocrine-gland-derived vascular endothelial growth factor; prokineticin 1; black mamba toxin-related protein