DATASHEET

Rat Prokineticin 1 / EGVEGF (PROK1) ELISA Kit

Catalogue No.:abx155461

Rat EG-VEGF ELISA Kit is a sandwich ELISA kit for use with Serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids. This assay has high sensitivity and excellent specificity for detection of Endocrine Gland Derived Vascular Endothelial Growth Factor (EG-VEGF)
No significant cross-reactivity or interference between Endocrine Gland Derived Vascular Endothelial Growth Factor (EG-VEGF) and analogues was observed.

Please note that this kit is also available as a CLIA Kit **abx190672**.

Target:	EG-VEGF
Reactivity:	Rat
Tested Applications:	ELISA
Recommended dilutions:	Optimal dilutions/concentrations should be determined by the end user.
Storage:	Shipped at 4 °C. Upon receipt, store the kit according to the storage instruction in the kit's manual.
Validity:	The validity for this kit is 6 months.
Stability:	The stability of the kit is determined by the rate of activity loss. The loss rate is less than 5% within the expiration date under appropriate storage conditions. To minimize performance fluctuations, operation procedures and lab conditions should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same user throughout.
Test Range:	15.62 pg/ml - 1000 pg/ml
Sensitivity:	< 6.5 pg/ml
Standard Form:	Lyophilized

DATASHEET

ELISA Detection:	Colorimetric
ELISA Type:	Sandwich
ELISA Data:	Quantitative
Sample Type:	Serum, plasma, tissue homogenates, cell lysates, cell culture supernatants and other biological fluids.
Target Type:	Antigen
Assay Principle:	This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient PROK1 will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to the PROK1 amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of PROK1 can be calculated.
Kit Components:	• Pre-coated 96-Well Microplate • Standard • Standard Diluent Buffer • Wash Buffer • Detection Reagent A • Detection Reagent B • Diluent A • Diluent B • TMB Substrate • Stop Solution • Plate Sealer
Material Required But Not Provided:	• 37°C incubator • Multi and single channel pipettes and sterile pipette tips • Squirt bottle or automated microplate washer • 1.5 ml tubes • Distilled water • Absorbent filter papers • 100 ml and 1 liter graduated cylinders • Microplate reader (wavelength: 450 nm) • ELISA Shaker

Sample Collection/Preparation:	• Serum: Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C. • Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C or -80°C. Avoid hemolysis and high cholesterol samples. • Tissue homogenates: The preparation of tissue homogenates will vary depending upon tissue type - this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
Reagent Preparation:	• 1) Standard: Prepare the standard with the recommended volume of Standard Diluent Buffer, to make the standard solution. Then use the Standard Diluent buffer to carry out serial dilutions of the standard solution, as instructed in the Protocol. • 2) Wash Buffer: Dilute the concentrated Wash Buffer with distilled water, as instructed in the Protocol. • 3) Detection Reagent Preparation: Calculate the total volume of working solution required. Dilute Detection Reagent A and Detection Reagent B with Diluent A and Diluent B, respectively, at 1:100.
Assay Procedure:	• 1) Set standard, test samples and control wells. • 2) Aliquot 100 µl of diluted standard into the standard wells. • 3) Aliquot 100 µl of Standard Diluent buffer into control (zero) well. • 4) Aliquot 100 µl of diluted samples into the sample wells. Incubate for 1 hr at 37 °C. • 5) Aliquot 100 µl of Detection Reagent A to each well. Incubate for 1 hr at 37 °C. • 6) Wash 3 times. • 7) Aliquot 100 µl of Detection Reagent B to each well. Incubate for 30 mins at 37 °C. • 8) Wash 5 times. • 9) Aliquot 90 µl of TMB Substrate to each well. Incubate for 10-20 mins at 37 °C. • 10) Aliquot 50 µl of Stop Solution. • 11) Measure the OD at 450 nm.

Protocol:

• Equilibrate the kit components and samples to room temperature (18 - 25 °C) before use. It is recommended to plot a standard curve for each test.
• 1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample at least in duplicate.
• 2. Add 100 µL of each standard, control and sample into the appropriate wells. Seal the plate with a cover and incubate for 1 h at 37°C.
• 3. Remove the cover and discard the liquid.
• 4. Add 100 µl of the detection Reagent A working solution to each well. Seal the plate with a cover and incubate for 1 h at 37°C.
• 5. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer.
• 6. Add 100 µL of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
• 7. Discard the solution and wash the plate 5 times with wash buffer as explained in previous step.
• 8. Aliquot 90 µl of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 10-20 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
• 9. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

Results Calculation:

For calculation, average the O.D.450 duplicate readings for each reference standard and each sample and substract the average control (zero) O.D.450 reading. The standard curve can be plotted as the relative O.D.450 of each reference standard solution (Y) vs. the respective concentration of each standard solution (X). The PROK1 concentration of the samples can be interpolated from the standard curve.

Assay Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of Prokineticin 1 / EGVEGF (PROK1)were were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of Prokineticin 1 / EGVEGF (PROK1) were tested on 3 different plates, 8 replicates in each plate.
CV (%) = (Standard Deviation / mean) × 100
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Note:

This product is for research use only.
The range and sensitivity is subject to change. Please contact us for the latest product information. For accurate results, sample concentrations must be diluted to mid-range of the kit. If you require a specific range, please contact us in advance or write your request in your order comments.
Please note that our ELISA and CLIA kits are optimised for detection of native samples, rather than recombinant proteins. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.