Progranulin (Human) ELISA Kit

(Catalog # K4738-100; 100 assays; Store at 4°C)

I. Introduction: Progranulin (PGRN) also called epithelin precursor, proepithelin (PEPI), PC cell-derived growth factor (PCDGF), acrogranin, or paragranulin is a 593aa cysteine-rich protein of 68.5kDa, that is typically secreted in a highly glycosylated 88kDa form. BioVision's Progranulin (human) ELISA kit is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human progranulin in biological fluids. A polyclonal antibody specific for progranulin has been precoated onto the 96-well microtiter plate. Standards and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, progranulin is recognized by the addition of a biotinylated polyclonal antibody specific for progranulin (Detection Antibody). After removal of excess biotinylated antibody, HRP labeled streptavidin (Detector) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of progranulin in the samples. The lowest level of progranulin that can be detected by this assay is 32 pg/ml. The Assay range is 0.063 ng/ml – 4 ng/ml. This ELISA is specific for the measurement of natural and recombinant human progranulin. It does not cross-react with human RBP4, human adiponectin, human Nampt, human leptin, human RELM-β, human ANGPTL6, human FABP4, human TNF-α, human IL-33, human GPX3, human resistin, human clusterin, human vaspin, human PAI1, human ANGPTL3, mouse Nampt, rat Nampt.

II. Sample Type: Serum, Plasma, Urine, Cell Culture Supernatant or CSF.

III. Kit Contents:

Components	K4738-100	Part No.
Plate coated with human Progranulin antibody	6 x 16-wells strips	K4738-100-1
Wash Buffer (10x)	30 ml x 2	K4738-100-2
ELISA Buffer (10X)	30 ml x 2	K4738-100-3
Detection Antibody (DET)	30 µl	K4738-100-4
HRP Labeled Streptavidin (lyophilized)	2 µg	K4738-100-5
Human progranulin Standard (lyophilized)	8 ng	K4738-100-6
TMB Substrate Solution	12 ml	K4738-100-7
Stop Solution	12 ml	K4738-100-8
Plate sealers (plastic film)	2	K4738-100-9
Silica Gel Minibags	2	K4738-100-10

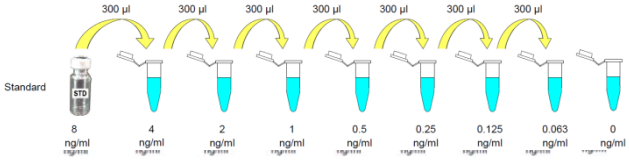
IV. User Supplied Reagents and Equipment:

- Microtiterplate reader at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Calibrated precision single and multi-channel pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

V. Storage and Handling: Reagent must be stored at 2-8°C when not in use. Plate and reagents should be at room temperature before use. Do not expose reagents to temperatures greater than 25°C.

VI. Reagent and Sample Preparation and Storage Conditions:

- Bring all reagents and samples to room temperature (18 - 25°C) before use.
- Wash Buffer 10X has to be diluted with deionized water 1:10 before use (e.g. 50 ml Wash Buffer 10X + 450 ml water) to obtain Wash Buffer 1X.
- ELISA Buffer 10X has to be diluted with deionized water 1:10 before use (e.g. 20 ml ELISA Buffer 10X + 180 ml water) to obtain ELISA Buffer 1X.
- Detection Antibody (DET) has to be diluted to 1:1000 in ELISA Buffer 1X (10 µl DET + 10 ml ELISA Buffer 1X).
  NOTE: The diluted Detection Antibody is not stable and cannot be stored.
- HRP Labeled Streptavidin (STREP-HRP) has to be reconstituted with 100 µl of ELISA Buffer 1X.
  - After reconstitution of STREP-HRP, prepare aliquots and store them at -20°C. Avoid freeze/thaw cycles.
  - Dilute the reconstituted STREP-HRP to the working concentration by adding 50 µl in 10 ml of ELISA Buffer 1X (1:200).
  - The diluted STREP-HRP is not stable and cannot be stored.
- Human Progranulin Standard (STD) has to be reconstituted with 1 ml of deionized water. This reconstitution produces a stock solution of 8 ng/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions. NOTE: The reconstituted standard is aliquoted and stored at -20°C
  - Dilute the standard protein concentrate (STD) (8 ng/ml) in Diluent 1X. A seven-point standard curve using 2-fold serial dilutions in Diluent 1X is recommended.
  - Suggested standard points are: 4, 2, 1, 0.5, 0.25, 0.125, 0.063 and 0 ng/ml.



FOR RESEARCH USE ONLY! Not to be used on humans.

- Sample Preparation:
  - Serum Samples: Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.
  - Plasma: Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.
  - Urine: Aseptically collect the urine of the day, voided directly into a sterile container. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze/thaw cycles.
  - Serum, Plasma, Urine, Cell Culture Supernatant have to be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use.
    NOTE: As a starting point, 1/200 dilution of serum or plasma and 1/20 dilution of urine are recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required! For CSF a starting dilution of 1/4-1/5 is recommended based on literature references using this ELISA Kit.

VII. Assay Protocol:

- Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips should be resealed in the foil pouch bag and stored at 4°C.
  NOTE: Remaining 16-well strips coated with progranulin antibody when opened can be stored at 4°C for up to 1 month.
- Add 100 µl of the different **standards** into the appropriate wells in duplicate. At the same time, add 100 µl of **diluted serum, plasma, urine, cell culture supernatant** samples in duplicate to the wells. Cover the plate with plate sealer and incubate for 1 hour at 37°C.
- Aspirate the coated wells and add 300 µl of **Wash Buffer 1X** using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
- Add 100 µl to each well of the **Detection Antibody.** Cover the plate with plate sealer and incubate for 1 hour at 37°C.
- Aspirate the coated wells and add 300 µl of **Wash Buffer 1X** using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
- Add 100 µl to each well of the diluted **HRP Labeled Streptavidin.** Cover the plate with plate sealer and incubate for 1 hour at 37°C.
- Aspirate the coated wells and add 300 µl of **Wash Buffer 1X** using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
- Add 100 µl to each well of **TMB Substrate Solution**. Allow the color reaction to develop at room temperature in the dark for 10 minutes.
- Stop the reaction by adding 100 µl of **Stop Solution.** Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
- Measure the OD at 450 nm in an ELISA reader within 30 minutes.

VIII. Calculation of Results:

- Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding progranulin concentration (ng/ml) on the vertical (Y) axis (see Typical Data).
- Calculate the progranulin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human progranulin in the samples.

IX. Typical Data:

The following data are obtained using the different concentrations of standard as described in this protocol:

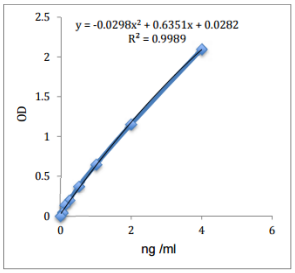


Figure: Standard curve

Standard hProgranulin (ng/ml)	Optical Density (mean)
4	2.097
2	1.1545
1	0.6445
0.5	0.3695
0.25	0.1905
0.125	0.134
0.0625	0.0415
0	0

X. RELATED PRODUCTS:

Progranulin, Human recombinant
Progranulin, Mouse recombinant
ELISA kits
Apoptosis Assay Kits and Reagents
Recombinant Growth Factors and Cytokines
Progranulin, Rat recombinant
Recombinant proteins and enzymes
Metabolism Assay kits and Reagents
Cellular Fractionation Kits
Polyclonal and monoclonal antibodies

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