



## User's Manual

# Human Progranulin ELISA Kit



DEIA-NB24-21



96T



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.

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## PRODUCT INFORMATION

### Intended Use

The Human Progranulin ELISA Kit is intended to be used for quantitative measurement of human progranulin in human serum and plasma samples for research use.

Allow measurements in cell culture media too and in

specimens others than serum e.g. in Cerebrospinal fluid, Amnion fluid, Saliva, Urine, Breast milk.

### General Description

Progranulin is also known as Granulin Epithelin Precursor, Proepithelin or Acrogranin. It is a 68.5 kDa protein, consisting of 593 amino acids (inclusive Signalpeptid), which appears in vivo in strongly glycosylated form and therefore has a size of approximately 90 kDa.

Progranulin has seven conserved domains, which are separated by linker sequences. By means of proteolytic cleavage, catalyzed by serine proteases like e.g. elastase, 6-25 kDa large fragments result, that are called Granulines or Epithelines. Progranulin is expressed and secreted in particular in strongly proliferating tissues such as adenoid tissue, spleen, skin epithelium, gastrointestinal mucous membranes, haematopoietic cells and in tumor cells. Until now no specific receptors, which would obtain the effect of Progranulin or the Granulines are known.

Progranulin seems to be a factor, which affects the wound healing positively. In case of skin lesions the expression is increased in ceratinocytes, in macrophages and in neutrophile cells. Progranulin affects the wound healing indirectly by activation of macrophages and stimulation of angiogenesis in the damaged tissue. The physiological effects of Progranulin and Granulines are oppositional. Progranulin can restrain TNF  $\alpha$  mediated pro-inflammatory processes. On the other hand the Granulines seem to stimulate the secretion of pro-inflammatory cytokines. The influence of Progranulin on inflammatory processes could be shown also in arteriosclerotic plaques. Here Progranulin is expressed by smooth muscle cells and affects the migration of monocytes and smooth muscle cells. In the central nervous system Progranulin is expressed in microglia and neurons (in neocortical and hippocampal pyramid cells as well as in purkinje cells in the cerebellum).

On mRNA level a clear increase of Progranulin expression could be shown during infections or injuries of the CNS, for example in mucopolysaccharidosis type I and IIIB, in viral inflammations of CNS, in amyotrophic lateral sclerosis and in Alzheimer's disease. Beyond that Progranulin seems to be of relevance in the development of sex specific differences during pre- and postnatal development and also for the neural plasticity in adults.

### Progranulin and Frontotemporal Dementia (FTD)

5-10 % of all dementias are of the frontotemporal form. A mutation in the gene for Progranulin (PGRN) could be shown in 5-10 % of the patients suffering FTD. Nearly all pathological mutations lead to a premature transcription interruption and to rapid degradation of the mutated mRNA. This results in a PGRN haploinsufficiency with clearly decreased Progranulin concentrations in serum. Due to these results several studies were accomplished, in order to clarify the suitability of Progranulin as marker for the PRGN dependent frontotemporal dementia. The results of these studies show that Progranulin can detect already presymptomatically a FTD. Due to the missing standardisation and the use of different antibodies in the commercially available test systems cut off value must be evaluated for each assay separately.

## Progranulin and Adiposity

Inflammatory processes are often increased in case of adiposity and type 2 diabetes, which is reflected by e.g. in the increase of the C-reactive Protein and pro-inflammatory cytokines e.g. IL-6. Youn et al. compared different groups of patients and have shown that the plasma concentration of Progranulin is significantly (1.4-fold) increased in type 2 diabetics compared to glucose-tolerant patients. The authors refer in particular to the positive correlation of the Progranulin concentration to the volume of the visceral adipose tissue. On the other hand no difference between slim and subcutaneous adipose patients has been detected in this study. For this reason the increase of the Progranulin concentration may reflect the body distribution of adipose tissue and thus represent a biomarker for visceral adipose tissue.

The Human Progranulin ELISA Kit is based on monoclonal antibodies, which detect with high specificity only Progranulin and not the single Granulines. Thus, a tool is available for the further investigation and validation of Progranulin as a biomarker for the visceral adipose tissue.

## Principles of Testing

The Human Progranulin ELISA Kit is a so-called Sandwich-Assay. It utilizes specific and high affinity monoclonal antibodies for this protein. The Progranulin in the samples binds to the immobilized first antibody on the microtiter plate. In the following step, the biotinylated antibody binds in turn to Progranulin. After washing, Streptavidin-Peroxidase-Enzyme conjugate will be added, which will bind highly specific to the biotin and will catalyse the enzymatic reaction, which turns the colour of the substrate, quantitatively depending on the Progranulin level of the samples.

## Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with human Progranulin antibody. Wells are separately breakable. (8x12) wells
2. **Standards (A-E):** lyophilized, contain recombinant Progranulin. Standard values are between 0.075 - 2.5 ng/ml (75, 250, 750, 1500 und 2500 pg/ml) Progranulin and have to be reconstituted with 1 ml (each) Dilution Buffer VP. Use 50 µl pro well in the assay.
3. **Dilution Buffer VP:** ready for use. Please use this for the reconstitution of Standards and Control Sera and for the dilution of Control Sera and Samples. Please shake before use! 1 x 50 mL
4. **Control Sera KS1 and KS2:** lyophilized, contain human Serum and should be reconstituted in each 250 µl Dilution Buffer VP. The Progranulin target values and the respective ranges are given on the certificate. The dilution should be according to the dilution of the respected samples. Use 50 µl per well in the assay. 1 x 250 µL
5. **Antibody Conjugate (AK):** ready for use, contains the biotinylated anti-Progranulin antibody. Use 50 µl for each well in the assay. 1 x 6 mL
6. **Enzyme Conjugate (EK):** contains HRP (Horseradish-Peroxidase)-labeled Streptavidin. Use 100 µl for each well in the assay. 1 x 12 mL
7. **Washing Buffer (WP):** 20-fold concentrated solution. Washing Buffer (WP) has to be diluted 1:20 with distilled or demineralised water before use (e.g. add the complete contents of the flask (50 ml) into a graduated flask and fill up with A. dest. to 1000 ml). Attention: After dilution the Washing Buffer is only 4 weeks stable, dilute only according to requirements. 1 x 50 mL
8. **Substrate (S):** ready for use, horseradish-peroxidase-(HRP) substrate. 1 x 12 mL

9. **Stopping Solution (SL):** ready for use, 0.2 M sulfuric acid. 1 x 12 mL
10. **Sealing Tape:** for covering the microtiter plate. 2

## Materials Required But Not Supplied

1. Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer WP (A. dest.).
2. Precision pipettes and multichannel pipettes with disposable plastic tips
3. Polyethylene PE/Polypropylene PP tubes for dilution of samples
4. Vortex-mixer
5. Microtiter plate shaker (350 rpm)
6. Microtiter plate washer (recommended)
7. Microplate reader ("ELISA-Reader") with filter for 450 and  $\geq 590$  nm

## Storage

Store the kit at 2-8°C after receipt until its expiry date. The lyophilized reagents should be stored at -20 °C after reconstitution. Avoid repeated thawing and freezing.

The shelf life of the components after initial opening is warranted for 4 weeks, store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The reconstituted components standards A-E and Control Sera KS1 and KS2 must be stored at -20°C (max. 4 weeks). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). The 1:20 diluted Washing Buffer WP is 4 weeks stable at 2-8°C.

## Specimen Collection And Preparation

### 1. Sample type: Serum and Plasma

No influence of 3.8 g/l Citrate, 5.4 mmol/l EDTA nor 30 IE/ml Heparin were shown on the measurement of Progranulin by the recovery experiments.

### 2. Sample stability

In firmly closable sample vials

- Storage at Room Temperature 20-25°C: max. 3 days
- Storage at 4°C: max. 3 days
- Storage at -20°C: 2 years

The measured values of serum and plasma samples did not show significant deviations up to 10 thaw/freezing cycles, values within the range of 95 to 101% of the target value were found.

### 3. Sample dilution

• Samples have to be diluted in Dilution Buffer (VP). For most of the determinations (serum or plasma samples, and no extreme values are expected) a serum or plasma dilution of 1:41 with Dilution Buffer VP should be suitable. According to expected Progranulin levels the dilution with VP can be higher or lower. The excellent linearity of this test system allows sample dilution of 1:20 to 1:320.

- Suggestion for dilution protocol: Pipette 400 µl Dilution Buffer VP in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 10 µl Serum- or Plasma (dilution 1:41). After mixing use 50 µl per determination of this dilution in the assay.

## Reagent Preparation

### 1. Standards and Controls

For the reconstitution of the lyophilised Standards A - E Dilution Buffer VP has to be used. The lyophilised Control Sera KS1 and KS2 must be reconstituted with the Dilution Buffer VP. The dilution should be according to the dilution of the respected samples. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam!) with a Vortex mixer. The reconstituted standards and controls can be stored for 1 month at -20°C. Repeated freeze/thaw cycles have to be avoided.

### 2. Washing Buffer

The required volume of Washing Buffer is prepared by 1:20 dilution of the provided 20-fold concentrate with deionised water. The diluted Washing Buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage!

### 3. Microtiter plate

Store the once unused microtiter strips and wells together with the desiccant in the tightly closed clip lock bag at 2-8°C use in the frame provided. The labelled expiry is not influenced in case of proper storage.

### 4. Substrate Solution

The Substrate Solution (S), stabilised Tetramethylbencidine, is photosensitive – store and incubate in the dark.

## Assay Procedure

### Note

1. Reagents with different lot numbers cannot be mixed. All reagents are stable until the indicated expiry, if stored unopened and protected from sunlight at 2-8°C.
2. The shelf life of the components after initial opening is warranted for 4 weeks.
3. Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming.
4. Incubation at room temperature means: Incubation at 20 - 25°C.  

The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must become adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations and/or false values, excessive shaking may result in high optical densities and/or false values.
5. Washing: Proper washing is of basic importance for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate

washing problems. All washing must be performed with the provided Washing Buffer WP diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least. The danger of handling with potentially infectious material must be taken into account.

**Automatic washing:** When using an Automatic microtiter plate washer, the respective instructions for use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

**Manual washing:** Washing Buffer may be dispensed via a multistep device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamical swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

- When performing the assay, Standards A-E, Control Sera KS1& KS2 and the samples should be pipetted as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, the Enzyme Conjugate EK as well as the succeeding Substrate Solution S should be added to the plate in the same order and in the same time interval as the samples. Stop Solution SL should be added to the plate in the same order as Substrate Solution S. All determinations (Standards, Control Sera and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

### Assay Step

- Add 50 µl Antibody Conjugate AK in all wells used.
- Pipette in positions A1/2 50 µl Dilution Buffer VP.
- Pipette in positions B1/2 50 µl of the Standard A (75 pg/ml),  
pipette in positions C1/2 50 µl of the Standard B (250 pg/ml),  
pipette in positions D1/2 50 µl of the Standard C (750 pg/ml),  
pipette in positions E1/2 50 µl of the Standard D (1500 pg/ml),  
pipette in positions F1/2 50 µl of the Standard E (2500 pg/ml).

To control the correct accomplishment of the assay 50 µl of the 1:41 (or in respective dilution ratio of the samples) in Dilution Buffer VP diluted Control Sera KS1/KS2 can be pipetted in positions G1/2 and H1/2.

Pipette 50 µl each of the diluted samples (e.g. dilute 1:41 with Dilution Buffer VP) in the rest of wells, according to your requirements.

- Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at  $\geq 350$  rpm)
- After incubation aspirate the contents of the wells and wash the wells 5 times 300 µl Washing Buffer WP / well.
- Following the last washing step pipette 100 µl of the Enzyme Conjugate EK in each well.
- Cover the wells with sealing tape and incubate the plate for 30 Minutes at room temperature (shake 350 rpm).

8. After incubation wash the wells 5 times with Washing Buffer WP as described in step 5.
9. Pipette 100 µl of the Substrate Solution S in each well.
10. Incubate the microtiter plate for 30 minutes in the dark at room temperature.
11. Stop the reaction by adding 100 µl Stopping Solution SL to all wells.
12. Measure the absorbance within 30 minutes at 450 nm (Reference filter  $\geq$  590 nm).

## Quality Control

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.3, these of standard E should exceed 0.8. Samples, which yield higher absorbance values than Standard E are beyond the standard curve, for reliable determinations these samples should be tested anew with a higher dilution.

## Calculation

1. Calculate the mean absorbance (MA) value for the blank from the duplicated determination (well A1/A2).
2. Subtract the mean absorbance (MA) of the blank from the mean absorbances of all other values.
3. Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4. Recommendation: Calculation of the standard curve should be done by using a computer program because the curve is in general (without respective transformation) not ideally described by linear regression. A higher-grade polynomial, or four parametric logistic (4-PL) curve fit or nonlinear regression are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).
5. The Progranulin concentration of the diluted sample or the diluted control sera in pg/ml (or ng/ml according the chosen unit for the standards) is calculated in this way, the Progranulin concentrations of the undiluted samples and of control sera are calculated by multiplication with the respective dilution factor.

### Exemplary calculation of Progranulin concentrations

Sample dilution: 1:41

Measured extinction of your sample 0.56

Measured extinction of Standard A 0.03

Your measurement program will calculate the Progranulin concentration of the diluted sample automatically by using the difference of sample and blank (0.03) for the calculation. You only have to determine the most suitable curve fit (here: polynomial 3 degree).

In this exemplary case the following equation is solved by the program to calculate the Progranulin concentration in the sample:

$$0.53 = 2.8552E-11x^3 - 1.0125E-7x^2 + 0.0010631x - 0.0033673$$

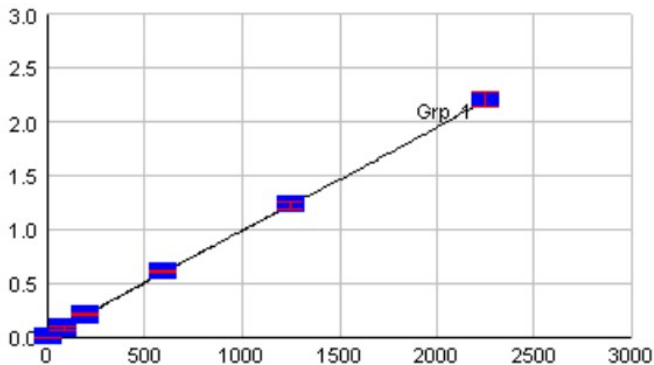
$$x = 0.5145$$

If the dilution factor (1:41) is taken into account the Progranulin concentration of the undiluted sample is

$$0.5145 \times 41 = 21.10 \text{ ng/mL}$$

## Typical Standard Curve

The exemplary shown standard curve in Figure below cannot be used for calculation of your test results. You have to establish a standard curve for each test you conduct!



## Precision

The inter and intra assay coefficients of variability are below 8.0 and 4.4 %, respectively.

Inter-Assay-Variation (results of 14 independent determinations)

	Mean value [ng/mL]	SD	CV [%]
Sample 1	36.78	2.49	6.76
Sample 2	23.40	1.87	7.99
Sample 3	21.52	1.37	6.36

Intra-Assay-Variation

	Determinations [n]	Mean value [ng/mL]	SD	CV [%]
Sample 1	19	25.61	0.87	3.38
Sample 2	19	49.74	2.17	4.35

## Sensitivity

The analytical sensitivity of the assay yields 0.018 ng/ml (pg/ml; as 2x SD of zero standard in 19-fold determination).

## Specificity

Commercially available sera from bovine, cat, chicken, dog, donkey, goat, guinea pig, horse, mouse, pig, rabbit, rat and sheep were diluted 1:5 und 1:41 and used as samples in this assay system and the signal intensity was measured. No cross reactivity was detected.

## Linearity

The Human Progranulin ELISA Kit is over a very wide range dilution authentic. The linearity of serum



dilutions is over a very wide range excellent.

Dilution	Sample 1 [ng/ml]	Sample 2 [ng/ml]	Sample 3 [ng/ml]
1:20	21.12	14.34	40.56
1:40	23.58	14.08	45.95
1:80	22.17	15.14	46.17
1:160	20.64	16.08	46.89
1:320	19.53	15.59	47.65
<b>AV / SD / CV%</b>	<b>21.41 / 1.54 / 7.20</b>	<b>15.05 / 0.84 / 5.57</b>	<b>45.44 / 2.81 / 6.18</b>

## Recovery

The recovery of recombinant Progranulin in serum and plasma samples varied from 91 to 101%.

## Interferences

Interference of physiological appearing substance with the Progranulin measurement was investigated. Serum samples have been enriched with different concentrations of possibly interfering substances and the amount of Progranulin was measured and compared with the Progranulin concentration in the same sample without any enrichment. In table below the relative results are shown. None of the tested substances interfered significantly with Progranulin measurement.

Triglyceride (100 mg/mL)	Bilirubin (200 µg/mL)	Hemoglobin (1 mg/mL)
104%	104%	117%

Effects of coagulation inhibitors were investigating by adding indicated amounts of inhibitors to VP or PBS enriched with 1250 pg/ml Progranulin. Relative amounts of Progranulin determined in inhibitor containing samples in comparison to inhibitor free samples are shown. None of the tested substances interfered significantly with Progranulin measurement.

3.8 g/l	Citrate	95%
5.4 mmol/l	EDTA	93%
30 IE/ml	Heparin	98%

## Precautions

1. For research and professional use only. The Creative Diagnostics kit is suitable only for in vitro use and not for internal use in humans and animals.
2. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Creative Diagnostics will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided.
3. Do not use obviously damaged or microbial contaminated or spilled material.
4. This kit contains material of human and/or animal origin. Therefore all components and patient's specimens should be treated as potentially infectious.

5. Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.
6. Following components contain human serum: Control Sera KS1, KS2

Source human serum for the Controls provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.