

ENG

Instructions for Use:
HUMAN PROGRANULIN ELISA

Catalogue number:
RMEE103R

For research use only!

 **BioVendor**
R&D[®]



BioVendor – Laboratorní medicína a.s.

Karásek 1767/1, 621 00 Brno, Czech Republic

+420 549 124 185

info@biovendor.com

sales@biovendor.com

www.biovendor.com

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HISTORY OF CHANGES

Previous version	Current version
ENG.005.A	ENG.006.A
Chapter 3 4) Control: The Progranulin target values and the respective ranges are given on the vial labels	Chapter 3 4) Control: The Progranulin target values and the respective ranges are given on the certificate.
Chapter 8 The shelf life of the components after opening is not affected, if used appropriately.	Chapter 8 The shelf life of the components after initial opening is warranted for 4 weeks.
Chapter 8.1 1 month	Chapter 8.1 2 months
Chapter 8.4 H ₂ O ₂ -Tetramethylbencidine	Chapter 8.4 Tetramethylbencidine

1. INTENDED USE

For quantitative detection of human Progranulin in serum and plasma sample.

- For research and professional use only!
- Is suited for Progranulin determination in **Serum** and **Plasma** samples.
- Is extremely **sensitive (18 pg/ml)** and, thus allows measurements in cell culture media too and in specimens others than serum e.g. in Cerebrospinal fluid, Amnion fluid, Saliva, Urine, Breast milk.
- Is **fast**: incubation time a total of 2 hours.
- Single Standards with **75, 250, 750, 1500, 2500 pg/ml** human Progranulin are provided in the Kit.
- 2 Control Sera are provided for quality control purposes according GLP.
- Is calibrated with **recombinant Progranulin**.
- Microtiter plates are separately breakapart, tests can be adapted to individual requirements.

2. INTRODUCTION

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 (1).

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 Epithelins. 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 (2, 3). 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 (4). The physiological effects of Progranulin and Granulines are oppositional. Progranulin can restrain TNF α mediated proinflammatory 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 (5). 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 (6).

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 humans suffering FTD (2). 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 PGRN dependent frontotemporal dementia (7, 8).

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 obese humans and have shown that the plasma concentration of Progranulin is significantly (1.4-fold) increased in type 2 diabetics compared to glucose-tolerant humans. 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 obese humans 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 (9).

The Progranulin ELISA RMEE103R 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.

3. REAGENTS PROVIDED

1	MTP	Microtiter plate , ready for use, with 96 wells, dived up in 12 stripes à 8 wells (separately breakapart), coated with human Progranulin antibody.
2	CAL	Standards A-E, lyophilised , 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	DILU	Dilution buffer VP, 50 ml , ready for use, after shaking. Please use this for the reconstitution of Standards and Control Sera and for the dilution of Control Sera and Samples .
4	Control	Control Sera KS1 and KS2, 250 µl , lyophilised, 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 pro well in the assay.
5	Ab	Antibody Conjugate AK, 6 ml , ready for use, contains the biotinylated anti-Progranulin antibody. Use 50 µl for each well in the assay.
6	CONJ	Enzyme Conjugate EK, 12 ml , ready for use, contains horseradisch-peroxidase conjugate to streptavidin, Use 100 µl for each well in the assay.
7	WASH BUF 20x	Washing Buffer (WP), 50 ml, 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.
8	SUBST	Substrate (S), 12 ml , ready for use, horseradish-peroxidase-(HRP)-substrate.
9	H ₂ SO ₄	Stopping Solution (SL), 12 ml , ready for use, 0.2 M sulphuric acid, Caution acid!
10		Sealing tape for covering of the microtiter plate, 2 x, adhesive.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Precision pipettes and multichannel pipettes with disposable plastic tips
- Distilled or deionized water for dilution of the Washing Buffer (WP)
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm
- Polyethylen PE/Polypropylen PP tubes for dilution of samples

5. WARNINGS AND PRECAUTIONS

The BioVendor kit is suitable only for in vitro use and not for internal use in humans and animals. Follow strictly the test protocol.

Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. BioVendor 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.

Do not use obvious damaged or microbial contaminated or spilled material.

5.1 Caution:

This kit contains material of human and/or animal origin. Therefore all components and patients specimens should be treated as potentially infectious.

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.

5.1.1 Human Serum

Following components contain human serum: **Control Sera KS1, KS2**

Source human serum for the control sera 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.

5.1.2 Reagents AK, EK, VP, WP, A-E

Contain as preservatives f 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

5.1.3 Substrate Solution (S)

The TMB-Substrate (S) contains 3,3',5,5' Tetramethylbenzidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.

5.1.4 Stopping Solution (SL)

The Stopping solution contains 0.2 M acid sulphur acid (H₂SO₄)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

5.2 General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

6. METHOD

The enzyme immunoassay for Progranulin RMEE103R 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.

7. SPECIMEN

Serum and plasma samples can be used in this assay. 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.

7.1 Storage of the samples

Storage at RT max. 3 days
Storage at +4°C max. 3 days
Storage at -20°C max. 2 years
in tightly closable plastic tubes.

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.

7.2 Sample Preparation

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 (see table 6).

Progranulin concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatants (see table 1).

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.

8. TECHNICAL RECOMMENDATIONS

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.

The shelf life of the components **after initial opening** is warranted for **4 weeks**

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.

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.

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 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.

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 is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamically 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.

8.1 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 2 months at –20°C. Repeated freeze/thaw cycles have to be avoided.

8.2 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!

8.3 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.

8.4 Substrate Solution

The Substrate Solution (S), stabilised H₂O₂-Tetramethylbenzidine, is photosensitive – store and incubate in the dark.

9. ASSAY PROCEDURE

When performing the assay, the 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 the 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.

1. Add 50 µl Antibody Conjugate AK in all wells used.
2. Pipette in positions A1/2 50 µl Dilution Buffer VP
3. 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.

4. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at ≥350 rpm)
5. After incubation aspirate the contents of the wells and wash the wells 5 times 300 µl Washing Buffer WP / well.
6. Following the last washing step pipette 100 µl of the Enzyme Conjugate EK in each well.
7. 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 ≥ 590 nm)

10. ESTABLISHING THE STANDARD CURVE

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.

The standards provided contain the following concentrations of Progranulin:

Standard	A	B	C	D	E
ng/ml	0.075	0.25	0.75	1.5	2.5
pg/ml	75	250	750	1500	2500

- 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 on semi-log paper (lin-log).
- 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 non-linear 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**.

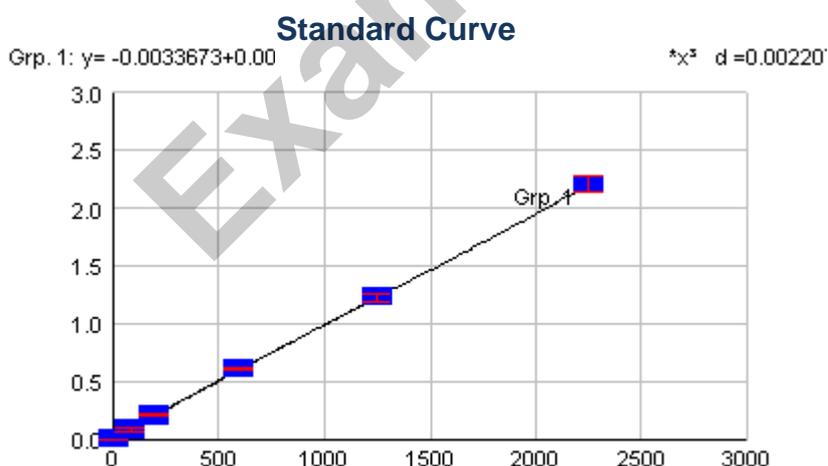


Figure 1. Exemplary Standard Curve with a polynomial 3 as curve fit.

The exemplary shown standard curve in Figure1 **cannot** be used for calculation of your test results. You have to establish a standard curve for each test you conduct!

Exemplary calculation of the Progranulin concentration of a 1:41 diluted sample:

Measured extinction of your sample	0.56
Measured extinction of the blank	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:

$$0.53 = -0.0033673 + 0.0010631x - 1.0125 \times 10^{-7} x^2 + 2,8552 \times 10^{-11} x x^3$$

$$0.5145 = x$$

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

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

11. PERFORMANCE CHARACTERISTICS

11.1 Standards

The standards are prepared from recombinant human Progranulin in concentrations of 75, 250, 750, 1500 and 2500 pg/ml (pico gram/ml, equal to 0.075 -2.5 nanogram/ml).

11.2 Sensitivity

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

11.3 Specificity

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

11.4 Recovery

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

11.5 Matrix effects

Matrix effects						
Dilution [1:x]	2	5	10	20	40	100
Saliva	> max.	> max.	102 %	-	-	-
Urine	106 %	102 %	107 %	-	-	-
Breast milk	> max.	108 %				
Cell culture media	69 %	81 %	91 %	104 %	-	-
Cerebrospinal fluid	73 %	88 %	93 %	-	-	-
Amnion fluid	> max.	> max.	> max.	> max.	102 %	100 %

- = not determined

Table 1: Matrix effects: % Recovery of recombinant Progranulin in different body fluids

11.6 Interference

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 2 the relative results are shown. None of the tested substances interfered significantly with Progranulin measurement.

	Triglycerides [100 mg/ml]	Bilirubin [200 µg/ml]	Haemoglobin [1 mg/ml]
%	104	104	117

Table 2: %- Recovery compared to non-enriched serum.

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.

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

Table 3: Effects of coagulation inhibitors.

None of the tested substances interfered significantly with Progranulin measurement.

11.7 Reproducibility and Precision

The inter and intra assay coefficients of variability are **below 8.0 and 4.4 %**, respectively. Exemplary determinations are shown in table 4 and table 5.

	Mean (ng/ml)	Standard deviation (ng/ml)	VC (%)
Sample 1	36.78	2.49	6.76
Sample 2	23.40	1.87	7.99
Sample 3	21.52	1.37	6.36

Table 4: Inter-Assay-Variation (results of 14 independent determinations)

	Number of determinations	Mean value (ng/ml)	Standard deviation (ng/ml)	VC (%)
Sample 1	19	25.61	0.87	3.38
Sample 2	19	49.74	2.17	4.35

Table 5: Intra-Assay-Variation (n=19)

11.8 Linearity

The Progranulin ELISA RMEE103R is over a very wide range dilution authentic. The linearity of serum dilutions is over a very wide range excellent (see table 6).

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
AV / 1SD / VC%	21.41 / 1.54 / 7.20	15.05 / 0.84 / 5.57	45.44 / 2.81 / 6.18

AV = Average Value, **SD**=Standard Deviation **VC** = Coefficient of Variation

Table 6: Linearity of the sample dilution (characteristic result of three different sera)

12. EXPECTATION VALUES

Concentrations of Progranulin human sera of 40 healthy adult donors, at the age of 20 to 65 were determined with the BioVendor ELISA RMEE103R. The concentrations of all samples varied from minimal 21.85 ng/ml to maximal 53.22 ng/ml (see table 7).

Gender	Number of samples	Median [ng/ml]	Average value [ng/ml]	Standard Deviation [ng/ml]	Min. – Max. [ng/ml]
female	20	32.22	31.60	5.62	21.85-40.57
male	20	30.71	33.06	8.11	22.27-53.22
total	40	31.32	32.33	17.35	21.85-53.22

Table 7: Expectation values for adults in serum

13. REFERENCES

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For more references on this product see our web pages at www.biovendor.com.

14. SUMMARY – PROGRANULIN ELISA RMEE103R

Reconstitution / Dilution of Reagents		
Standards A-E	Reconstitution in Dilution Buffer VP	1 ml each
Control Serum KS1 & KS2	Reconstitution in Dilution Buffer VP	250 µl each
Washing Buffer WP	dilute in A. dest. (e.g. add the complete contents of the flask 50 ml into a graduated flask and fill with A.dest. to 1000 ml)	1:20
Sample Dilution + Control Sera KS1 & KS2: 1:41 in Dilution Buffer VP, mix directly and use within max. 60 min. Use 50 µl per determination		
Before assay procedure bring all reagents to room temperature		

Example Version

15. ASSAY PROCEDURE FOR DOUBLE DETERMINATIONS

Pipette	Reagent	Position
50 µl	Antibody Conjugate AK	in all wells used
50 µl	Dilution Buffer VP (blank)	A1 and A2
50 µl	Standard A (75 pg/ml)	B1 and B2
50 µl	Standard B (250 pg/ml)	C1 and C2
50 µl	Standard C (750 pg/ml)	D1 and D2
50 µl	Standard D (1500 pg/ml)	E1 and E2
50 µl	Standard E (2500 pg/ml)	F1 and F2
50 µl	Control Serum KS1	G1 and G2
50 µl	Control Serum KS2	H1 and H2
50 µl	Samples	following wells
Cover the wells with the sealing tape.		
Incubation: 1 h at RT, 350 rpm		
5 x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl Wash Buffer WP	each well
100 µl	Enzyme Conjugate EK	each well
Incubation: 30 min at RT, 350 rpm		
5 x 300 µl	Aspirate the contents of the wells and wash 5x with 300 µl Wash Buffer WP	each well
100 µl	Substrate S	each well
Incubation: 30 min in the dark RT		
100 µl	Stop Solution SL	each well
Measure the absorbance within 30 min at 450 nm with \geq 590 nm as reference wavelength.		

REF RMEE103R



International Test Description

	CAL A-E	A - E	Rec in 1 ml VP	
	Control	KS1&KS2	Rec in 250 µl VP	
	WASHBUF 20x	WP		1:20 DILU A. dest.
		Control		1:41 DILU VP
		SPE		1:41 DILU VP
20-25 °C				
50 µl		Ab		A1 - End
50 µl		BUF VP		A1/2
50 µl		CAL A (75 pg/ml)		B1/2
50 µl		CAL B (250 pg/ml)		C1/2
50 µl		CAL C (750 pg/ml)		D1/2
50 µl		CAL D (1500 pg/ml)		E1/2
50 µl		CAL E (2500 pg/ml)		F1/2
50 µl	CONTROL KS 1	1:41	DILU VP	G1/2
50 µl	CONTROL KS 2	1:41	DILU VP	H1/2
50 µl	SPE	1:41	DILU VP	
			TAPE	
1 h 20-25 °C ↔ 350 rpm				
5 x 300 µl			5 x WASHBUF WP	
100 µl			CONJ	
			TAPE	
0.5 h 20-25 °C ↔ 350 rpm				
5 x 300 µl			5 x WASHBUF WP	
100 µl			SUBST TMB S	
30 min 20-25 °C 				
100 µl			H₂SO₄ SL	
			MEASURE	

16. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
 <p data-bbox="260 1184 467 1216">www.biovendor.com</p>	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks



BioVendor – Laboratorní medicína a.s.

Karásek 1767/1, 621 00 Brno, Czech Republic

+420 549 124 185

info@biovendor.com

sales@biovendor.com

www.biovendor.com

