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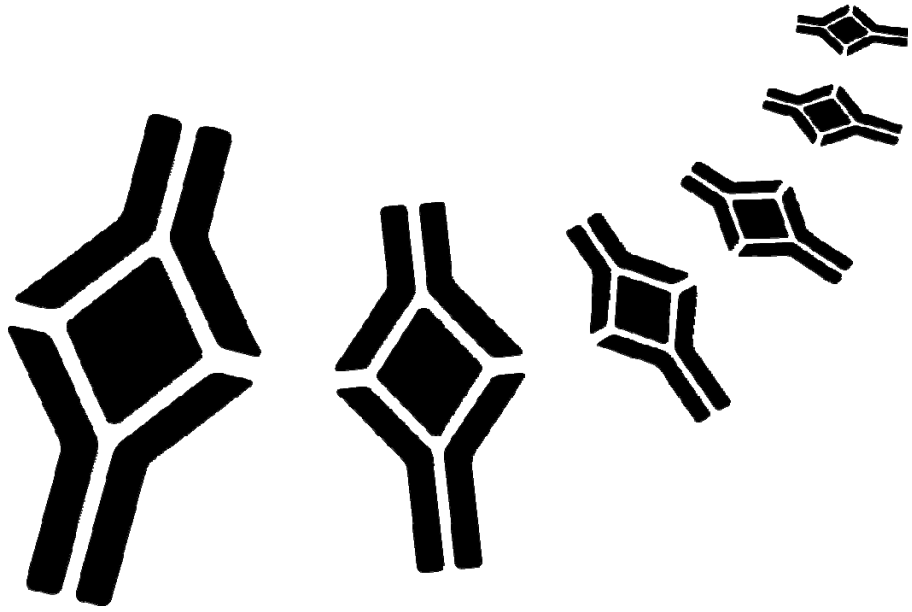

HUMAN PROGRANULIN ELISA

Product Data Sheet

Cat. No.: RMEE103R

For Research Use Only

VERSION 51 050510

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

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

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 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 (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 TNFα 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 (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).

2.1 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 (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 PRGN dependent frontotemporal dementia (7, 8).

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.

2.2 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 (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 à8 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 vial labels. 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	WASHBUF 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, stabilised H_2O_2 Tetramethylbencidine.
9	H_2SO_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 (100 and 200 µl) Micropipettes and multichannel pipettes with disposable plastic tips
- Distilled or Deionized water for dilution of the Washing Buffer (WP)
- Vortex-mixer
- Device to aspirate the standards and the samples from the wells (recommended because of the potential danger of infection by human samples)
- Timer (120 min. range)
- Reservoirs (disposable)
- Plate washer and plate shaker (recommended)
- Calibrated Micro plate reader ("ELISA-Reader") with filter for 450 and 620 nm (or ≥590 nm)

5. WARNINGS AND PRECAUTIONS

For in-vitro diagnostic use only. For professional use only.
Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
Caution: This kit contains material of human and/or animal origin. Source human serum for the Control Serum provided in this kit was tested by FDA recommended methods and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibodies. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.
Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step. Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
Do not use expired reagents.

2-Methyl-4-Isothiazolin-3-one

Following components contain < 0.01% **2-Methyl-4-isothiazolin-3-one** solution as preservative **A-E, AK, EK, VP**

< 0.01% 2-Methyl-4-isothiazolin-3-one Solution

R36/38 Irritating to eyes and skin

R43 Sensibilisation through skin contact possible

S26 In case of contact with eyes rinse immediately with plenty of water and seek medical advice

S28.1 After contact with skin wash immediately with plenty of water

5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one

Following components contain < 0.01%(w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-isothiazol-3-one as preservative: **A-E, AK, EK, VP, WP**

R36/38 Irritating to eyes and skin

R43 Sensibilisation through skin contact possible

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice S28.1 S28.1 After contact with skin, wash immediately with plenty of water

Stop solution contains 0.2 M Sulfuric Acid (H_2SO_4)

R36/38 Irritating to eyes and skin

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S28.1 After contact with skin, wash immediately with plenty of water

S36/37 Wear suitable protective clothing and gloves.

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine. Store and incubate in the dark.

R20/21/R22 Harmful by inhalation, in contact with skin and if swallowed

R36/37/38 Irritating to eyes, respiratory system and skin

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S28.1 After contact with skin, wash immediately with plenty of water

S36/37 Wear suitable protective clothing and gloves

General first aid procedures:

Skin contact: Wash affected area thoroughly with water. Discard 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: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.

Do not eat, drink or smoke in these areas.

Never pipette the materials with the mouth.

Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

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 ml Dilution Buffer VP** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **10 µl Serum- or Plasma** (dilution1:41). After mixing use 50 µl per determination of this dilution in the assay.

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.

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: 20-25°C

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 H_2O_2-Tetramethylbencidine, 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 ng/ml),**
pipette in positions C1/2 **50 µl of the Standard B (250 ng/ml)**,
pipette in positions D1/2 **50 µl of the Standard C (750 ng/ml)**,
pipette in positions E1/2 **50 µl of the Standard D (1500 ng/ml)**,
pipette in positions F1/2 **50 µl of the Standard E (2500 ng/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 **250 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** (if possible 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; e.g. 620 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**.

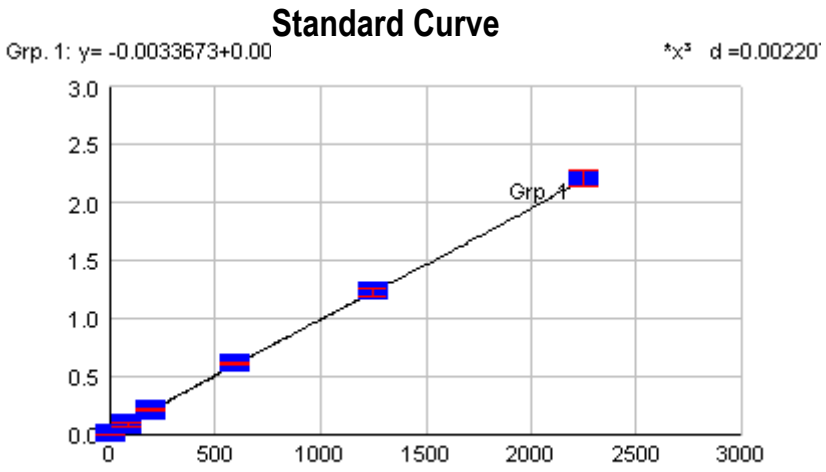

Figure 1. Exemplary Standard Curve with a polynomial 3 as curve fit.

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:

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

$0.5145 = x$

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

$0.5145 \times 41 = 21.10$ 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 nano gram/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 und 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

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

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

- = not determined

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.

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

	Triglycerides [100 mg/ml]	**Bilirubin** [200 µg/ml]	**Haemoglobin** [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.

Table 3: Effects of coagulation inhibitors.

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

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.

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

	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 5: Intra-Assay-Variation

	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

11.8 Linearity

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

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

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

12. EXPECTATION VALUES

Concentrations of Progranulinin 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).

Table 7: Expectation values for adults in serum

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

13. REFERENCES

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» **For more references on this product see our WebPages 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**		

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 250 µl	Aspirate the contents of the wells and wash **5x** with **250 µl Wash Buffer WP**	each well
100 µl	Enzyme Conjugate **EK**	each well

Incubation: 30 min at **RT**, **≥350 rpm**

5 x 250 µl	Aspirate the contents of the wells and wash **5x** with **250 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 **≥ 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**
°C **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 °C 20-25 ≥ 350 rpm

5 x 250 µl	**5 x WASHBUF WP**
100 µl	**CONJ**
	TAPE

0.5 h °C 20-25 ≥ 350 rpm

5 x 250 µl	**5 x WASHBUF WP**
100 µl	**SUBST TMB S**

30 min °C 20-25

100 µl	**H_2SO_4 SL**
	MEASURE

NOTES

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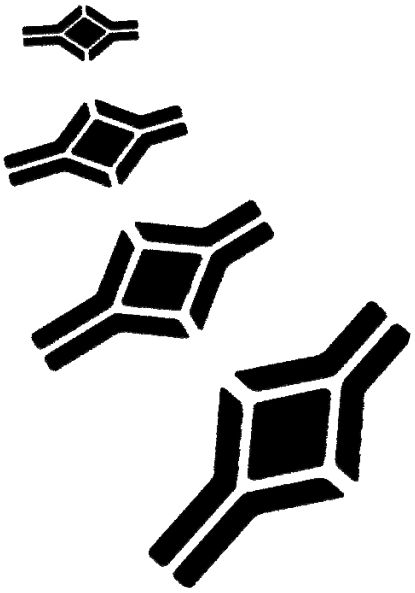

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