Progranulin ELISA

For the quantitative determination of progranulin in human serum and plasma.

For Research Use Only. Not for Diagnostic Procedures.

Catalog Number: 22-PRGHU-E01
Size: 96 Wells
Version: 26.03.2015 V5 – ALPCO 1.0

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1. Intended Use

The Progranulin ELISA is designed for the quantitative determination of progranulin in human serum and plasma. For Research Use Only. Not for use in diagnostic procedures.

2. Principle of the Assay

The Progranulin ELISA is a sandwich assay. It utilizes specific and high affinity monoclonal antibodies for this protein. The progranulin in the samples binds to the immobilized primary antibody on the microtiter plate. In the following step, the biotinylated antibody binds to progranulin. After washing, Streptavidin-Peroxidase-Enzyme conjugate is added, which binds the biotin with high specificity. The enzyme reacts with substrate to produce a blue color that changes to yellow upon addition of substrate. Color generation is proportional to the progranulin level in the samples.

3. Warnings and Precautions

This kit is suitable for research use only and not for internal use in humans and animals. Strictly follow the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. ALPCO 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 obviously damaged, microbial contaminated, or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore, all components and samples 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.

Human Serum
The 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 samples should be treated as potentially infectious.

Reagents AK, EK, VP, WP, A-E
Contain as preservatives **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/ vapors/ 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.

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/ vapors/ 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.

Stop Solution (SL)

The Stop solution contains 0.2 M sulfuric acid (H_2SO_4)

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.

3.1 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. To assure an effective rinse, 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.

4. Sample Handling and Preparation

Serum and plasma samples can be used in this assay. Samples must 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. Sample dilutions ranging from 1:20 to 1:320 are supported. (see Table 6).

Progranulin concentrations in body fluids other than serum or cell culture supernatants may be quite different (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 well of this dilution in the assay.

Interferences

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 (see Table 3).

Storage of the samples
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.

Storage at RT max. 3 days
Storage at +4°C max. 3 days
Storage at –20°C max. 2 years

5. Materials

5.1 Materials Provided

The reagents listed below are sufficient for 96 wells including the controls.

MTP	**Microtiter plate**: Microtiter plate with 96 wells, divided into 12 removable strips with 8 wells each, coated with human Progranulin antibody.
STD	**Standards A-E,** lyophilized, contain recombinant Progranulin. Values between 0.075 – 2.5 ng/ml (75 – 2500 pg/ml) Progranulin. Reconstitute with 1 ml (each) Dilution Buffer VP. Use 50 µl/well in the assay.
BUF	**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**.
Control	**Control Sera KS1 and KS2**, **250 µl,** lyophilized, contain human Serum. **Reconstitute each with 250 µl Dilution Buffer VP**. The Progranulin target values and respective ranges are given on the vial labels. The dilution should be according to the dilution of the respected samples. Use **50 µl/**well in the assay.
Ab	**Antibody Conjugate AK, 6 ml**, ready for use, contains the biotinylated anti-Progranulin antibody. Use 50 µl for each well in the assay.
CONJ	**Enzyme Conjugate EK, 12 ml**, ready for use, contains horseradish-peroxidase conjugate to streptavidin. Use 100 µl for each well in the assay.
WASHBUF 20x	**Wash Buffer (WP), 50 ml, 20X Concentrate**. **Wash Buffer (WP)** has to be diluted 1:20 with distilled or deionized water before use (e.g. add the complete contents of the flask (50 ml) into a graduated flask and fill up with deionized water to 1000 ml). Attention: After dilution, the Wash Buffer is only stable for 4 weeks, dilute only according to requirements.
SUBST	**Substrate (S), 12 ml**, ready for use, horseradish-peroxidase-(HRP)-substrate
H_2SO_4	**Stop Solution (SL), 12 ml,** ready for use, 0.2 M sulfuric acid, Caution acid!
	Sealing tape: for covering of the microtiter plate, 2 x, adhesive

5.2 Materials not Provided

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

6.0 Technical Notes

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 opening is not affected, if used appropriately.

Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitates in the buffers must be dissolved before usage by mixing and/or warming.

Incubation at room temperature means: Incubation at 20-25°C

The incubation steps are recommended to be performed at 350 rpm. Differences in plate shaker models may require an adjustment of the shake rate. 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 random variations of measured optical densities, potentially leading to falsely calculated sample values. Effects like high background values or high variations may indicate washing problems.

All washing must be performed with the provided wash buffer diluted to usage concentration. Wash volume per wash cycle and well must be at least 300 µl.

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. Following the last aspiration step of each washing cycle, the fluid remaining in each well should be removed by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

Manual washing is an adequate alternative option. Wash Buffer may be dispensed with a squirt bottle. The fluid may be removed by dynamically swinging out the microtiter plate over a basin. If aspirating devices are used, care must be taken that the inside well surface is not scratched. After every single wash step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on lint-free absorbent tissue.

Standards and Controls

The lyophilized **Standards A – E** must be reconstituted with 1 ml each of **Dilution Buffer VP**. **Control Sera KS1 and KS2** must be reconstituted with 250 µl each of **Dilution Buffer VP.** 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 up to 2 months at –20°C. Repeated freeze/thaw cycles must be avoided.

Wash Buffer

The required volume of Wash Buffer is prepared by 1:20 dilution of the 20x Wash Buffer concentrate with deionized water. The diluted Wash Buffer is stable for 4 weeks at 2-8°C. It must be at room temperature for usage.

Microtiter plate

Store unused microtiter strips and wells together with the desiccant in the tightly closed clip lock bag at 2-8°C in the frame provided. If properly stored, the plate is stable until the labelled expiry.

Substrate Solution
The Substrate Solution (S), stabilized H_2O_2-Tetramethylbenzidine, is photosensitive – store and incubate in the dark.

The danger of handling with potentially infectious material must be considered.

7. Procedure

Test Protocol
When performing the assay, the **Standards A-E, Control Sera KS1& KS2,** and the samples should be pipetted as quickly as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times, the **Enzyme Conjugate EK** as well as the **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 50 µl **Dilution Buffer VP** in wells A1/2.
3) Pipette:
   50 µl of Standard **A** (75 pg/ml) in wells B1/2
   50 µl of Standard **B** (250 pg/ml) in wells C1/2
   50 µl of Standard **C** (750 pg/ml) in wells D1/2
   50 µl of the Standard **D** (1500 pg/ml in wells E1/2
   50 µl of Standard **E** (2500 pg/ml) in wells F1/2.
   Pipette 50 µl of the 1:41 **Control Sera KS1/KS2** in wells G1/2 and H1/2.
   Pipette 50 µl of the diluted samples (e.g., dilute 1:41 with Dilution Buffer VP) in the rest of wells, according to 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 using 300 µl **Wash 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 **Wash 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 **Stop Solution SL** to all wells.
12) Measure the absorbance at 450 nm (reference filter ≥ 590 nm) within 30 minutes of addition of stop solution.

8. Results

For the evaluation of the assay, the absorbance value of the blank should be below 0.3, and standard E should be greater than 0.8. Samples with higher absorbance values than Standard E are beyond the standard curve and should be retested with a greater dilution for reliable determination.

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 value for the blank from the duplicate determination (well A1/A2).
2) Subtract the mean absorbance of the blank from the mean absorbance 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 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.
5) The Progranulin concentration of the diluted sample or the diluted control sera in pg/ml (or ng/ml if selected as the 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.

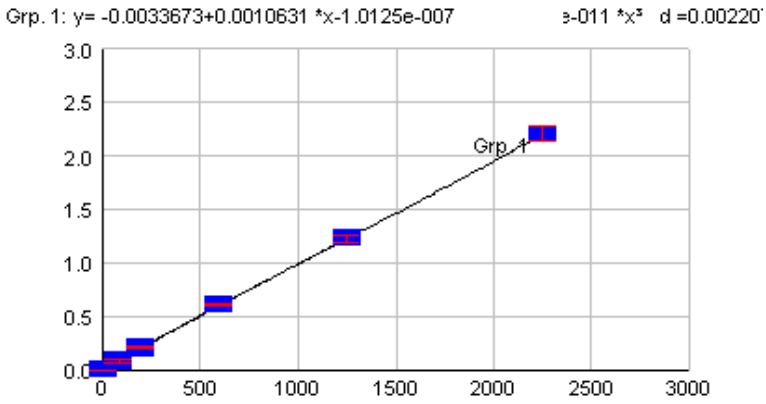

Figure 1. Exemplary Curve with a 3rd Order Polynomial Fit

The example standard curve shown in Fig.1 **cannot** be used for calculation of test results. A standard curve must be established for each test conducted.

Example 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

A 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. Users only have to determine the most suitable curve fit (here: 3rd degree polynomial).

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

$0.53 = -0.0033673+0.0010631x-1.0125\times10^{-7}\,x^2+2.8552\times10^{-11}\times x^3$
$0.5145 = x$

If the dilution factor (1:41) is considered, the Progranulin concentration of the undiluted sample is:
0.5145 × 41 = 21.10 ng/ml

9. Performance Characteristics

Standards
The standards are prepared from recombinant human Progranulin in concentrations of 75, 250, 750, 1500 and 2500 pg/ml (0.075 -2.5 ng/ml)

Sensitivity
The analytical sensitivity of the assay is 0.018 ng/mL (18 pg/mL) [determined as 2SD of 19 measurements of the zero standard]

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.

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

Matrix Effects

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

Dilution	Saliva	Urine	Breast Milk	Cell Culture Media	Cerebrospinal Fluid	Amniotic Fluid
1:2	>max.	106%	>max	69%	73%	>max
1:5	>max.	102%	>max	81%	88%	>max
1:10	102%	107%	>max	91%	93%	>max
1:20	-	-	>max	104%	-	>max
1:40	-	-	>max	-	-	102%
1:100	-	-	108%	-	-	100%
Dilution	**Saliva**	**Urine**	**Breast Milk**	**Cell Culture Media**	**Cerebrospinal Fluid**	**Amniotic Fluid**
1:2	>max.	106%	>max	69%	73%	>max
1:5	>max.	102%	>max	81%	88%	>max
1:10	102%	107%	>max	91%	93%	>max
1:20	-	-	>max	104%	-	>max

1:40	-	-	>max	-	-	102%
1:100	-	-	108%	-	-	100%

"-" = not determined

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

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

Effects of coagulation inhibitors were investigated 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
Citrate (3.8 g/L)	95
EDTA (5.4 mmol/L)	93
Heparin (30 IE/mL)	98

Reproducibility and Precision
The inter- and intra-assay coefficients of variability are below 8.0 and 4.4 %, respectively. Example 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)**	**% 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

Table 5: Intra-Assay-Variation (results of 19 independent determinations).

	Mean Value (ng/mL)	Standard Deviation (ng/mL)	%CV
Sample 1	25.61	0.87	3.38
Sample 2	49.74	2.17	4.35

Linearity
The Progranulin ELISA works over a very wide range of dilutions. The linearity of three separate serum samples are found below.

Table 6. Linearity Data

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
Average	21.41	15.05	45.44
1 SD	1.54	0.84	2.81
%CV	7.20	5.57	6.18

10. Literature

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7. Finch N, Baker M, Crook R, Swanson K, Kuntz K, Surtees R, et al. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. Brain 2009;132:583-91.
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11. Summary

Reagent Preparation:	**Dilution:**	
Standards A-E	Reconstitution in **Dilution Buffer VP**	1 mL each
Control Serum KS1 & KS2	Reconstitution in **Dilution Buffer VP**	250 µL each
Wash Buffer WP	Dilute in deionized water (e.g. add the complete contents of the flask 50 ml into a graduated flask and fill with DI water to 1000 ml)	1:20
Sample Dilution + Control Sera KS1 & KS2: 1:41 in Dilution Buffer VP mix directly and use within 60 minutes. Use 50 µL per determination.		
Before assay procedure bring all reagents to room temperature.		

Assay Procedure: Testing in Duplicate

Pipette	**Reagents**	**Position**
50 µL	Pipette Antibody Conjugate **AK**	In all wells
50 µL	Add Dilution Buffer **VP** (blank)	A1/A2
50 µL	Standard **A (75 pg/mL)**	B1/B2
50 µL	Standard **B (250 pg/mL)**	C1/C2
50 µL	Standard **C (750 pg/mL)**	D1/D2
50 µL	Standard **D (1500 pg/mL)**	E1/E2
50 µL	Standard **E (2500 pg/mL)**	F1/F2
50 µL	Control Serum **KS1**	G1/G2
50 µL	Control Serum **KS2**	H1/H2
50 µL	Samples	Following wells
Cover the wells with the sealing tape.		
Incubation: 1 hr at RT, 350 rpm		
5 x 300 µL	Aspirate the contents of the wells and wash 5X with 300 µL/well **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/well **Wash Buffer WP**	Each well
100 µL	Substrate Solution **S**	Each well
Incubation: 30 min in the Dark at RT		
100 µL	Stop Solution **SL**	Each well
Measure the absorbance within 30 minutes at 450 nm (Reference Wavelength ≥ 590 nm)		