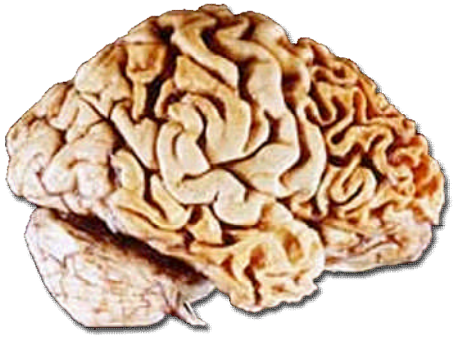
Human Progranulin
ELISA E103

Progranulin (Granulin Epithelin Precursor, Pro-epithelin or Acrogranin) is a 68.5 kDa protein, of 593 amino acids (inclusive signal peptide), which appears in vivo strongly glycosylated and therefore has a size of approximately 90 kDa. Progranulin is mainly expressed and secreted in strongly proliferating tissues. Progranulin expression has been detected in different cell types e.g. microglia, pyramidial cells, purkinje cells and smooth muscle cells. Proteolytic cleavage by serine proteases results in several granulines called peptides.



Progranulin seems to be a factor, which affects the wound healing positively by activation of macrophages and stimulation of angiogenesis in the damaged tissue. The physiological effects of Progranulin and Granulines are oppositional:

Progranulin: anti-inflammatory
Granulines: pro-inflammatory

Progranulin and Frontotemporal Dementia (FTD)
A mutation in the progranulin gene (PGRN) has been detected in 5-10% of FTD patients. This mutation results in a PGRN haploinsufficiency with clearly decreased progranulin concentrations in serum. Several studies demonstrated FTD can be detected pre-symptomatically by progranulin measurement.

Progranulin and Adiposity
Inflammatory processes are often increased in case of adiposity or type 2 diabetes and it was shown that the **plasma concentration of Progranulin is significantly (1.4-fold) increased in type 2 diabetics**. Further, Progranulin concentration seemed to be positively correlated with the volume of the visceral adipose tissue. Thus, Progranulin concentration may reflect the distribution of adipose tissue and represent a **biomarker for visceral adipose tissue**.

Assay Characteristics
Mediagnost Progranulin ELISA E103

The Mediagnost Progranulin ELISA E103 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.**

- ✓ human Progranulin separate standards: 75-2500 pg/ml
- ✓ Analytical sensitivity of 18 pg/ml
- ✓ High Precision:
  Intra-Assay Variance of < 4.4%
  Inter-Assay Variance of < 8.0%
- ✓ 2 Control Sera for GLP conformity
- ✓ Fast: Incubation time of 2 hours

Linearity human Progranulin ELISA E103 [ng/ml]			
Dilution	**Sample 1**	**Sample 2**	**Sample 3**
1:20	14.34	21.12	40.56
1:40	14.08	23.58	45.95
1:80	15.14	22.17	46.17
1:160	16.08	20.64	46.89
1:320	15.59	19.53	47.65

Specimen
Serum and plasma samples can be used in this assay. No influence of 3.8 g/l Citrat, 5.4 mmol/l EDTA or 30 IE/ml Heparin on the measurement of Progranulin have been detected by recovery experiments.

Stability of the Samples
Storage at 4°C up to 3 days
Storage at 25°C up to 3 days
Not more than 3 freeze/thaw cycles!

Matrix effects: Recovery of recombinant Progranulin						
Dilution [1:x]	**2**	**5**	**10**	**20**	**40**	**100**
Saliva	> max.	> max.	102 %	-	-	-
Urine	106 %	102 %	107 %	-	-	-
Breast milk	> max.	> max.	> max.	> max.	> max.	108 %
Cell culture Media	69 %	81 %	91 %	104 %	-	-
Cerebro-spinal fluid	73 %	88 %	93 %	-	-	-

- = not determined

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Mediagnost human Progranulin ELISA E103

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**		

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

5x 300µl	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

5x 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 **≥ 590 nm** as reference wavelength.		

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