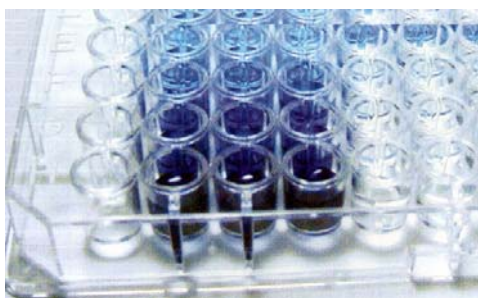


Human Progranulin ELISA Kit

Cat. No. P0731EK



Instruction Manual
Version 1.0.0

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES



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Introduction

The granulins are a family of cysteine-rich polypeptides, some of which have growth modulatory activity. The widespread occurrence of granulin mRNA in cells from the hematopoietic system and in epithelia implies important functions in these tissues. All 4 known human granulin-like peptides are encoded in a single precursor, progranulin, which has a highly conserved 12-cysteine backbone defining a consensus sequence that is repeated 7 times (1).

Progranulin is a 593-amino acid glycoprotein, the mRNA of which is expressed in many epithelial cells both in vitro and in vivo. He and Bateman demonstrated that overexpression of the progranulin gene in SW-13 adrenal carcinoma cells and MDCK nontransformed renal epithelia resulted in transfection-specific secretion of progranulin, acquired clonogenicity in semisolid agar, and increased mitosis in monolayer culture, whereas diminution of progranulin gene expression impaired growth of these cells (2). They proposed that the rate of growth of some epithelia is proportional to the level of intrinsic progranulin gene expression, and that elevated progranulin gene expression confers a transformed phenotype on epithelial cells including anchorage independence in vitro and growth as tumors in nude mice. They also found that in murine transcutaneous puncture wounds, progranulin mRNA is expressed in the inflammatory infiltrate and is highly induced in dermal fibroblasts and endothelia following injury. When applied to a cutaneous wound, progranulin increased the accumulation of neutrophils, macrophages, blood vessels, and fibroblasts in the wound. It acted directly on isolated dermal fibroblasts and endothelial cells to promote division, migration, and the formation of capillary-like tubule structures, concluding that progranulin is, therefore, probably a wound-related growth factor (3). Due to its tumor-promoting activity and multiple functions on hematopoietic cells and endothelial cells, progranulin can be a novel marker for oncology and hematology.

Assay Principles

This kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of progranulin in human serum, plasma or cell culture supernatants.

A polyclonal antibody specific for human progranulin has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any progranulin present is bound by immobilized antibody. Bound progranulin is captured by biotinylated anti-human progranulin polyclonal antibody. HRP conjugated streptavidin is added. After washing, a substrate solution is added. The colors develop in proportion to the bounded progranulin quantity. The color development is stopped and the intensity of color is measured.

Kit Components

- 1) Antibody coated 96-well plate, 12 X 8-well strips
- 2) 5x Wash concentrate, 100 ml
- 3) 5x Diluent, 50 ml
- 4) Secondary antibody, 12 ml
- 5) 100x Detector, 150 μ l
- 6) Standard, recombinant human progranulin expressed by HEK 293 cells, 1 vial, lyophilized
- 7) QC sample = recombinant human progranulin protein, 1 vial, lyophilized (For actual concentrations of QC sample, see the 'Certificate of analysis' enclosed.)
- 8) Substrate, 12 ml
- 9) Stop solution, 12 ml
- 10) Plate sealer, 3 sealers

Reagents Description

Antibody coated 96-well plate, 12 x 8-well strips, with absorbed polyclonal antibody against human progranulin

5x Wash concentrate, buffered detergent solution, supplied as a 5x concentrate

5x Diluent, for sample and reagent dilution

1x Secondary antibody, biotinylated polyclonal antibody against human progranulin
100x detector, HRP conjugated streptavidin
Standard, 8.0 ng, 1 vial each, recombinant human progranulin, lyophilized
QC sample, 1 vial each, recombinant human progranulin protein
Substrate, chromogenic reagents
Stop solution, 1M H₃PO₄

Storage of Reagents

Reagent must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but not Supplied

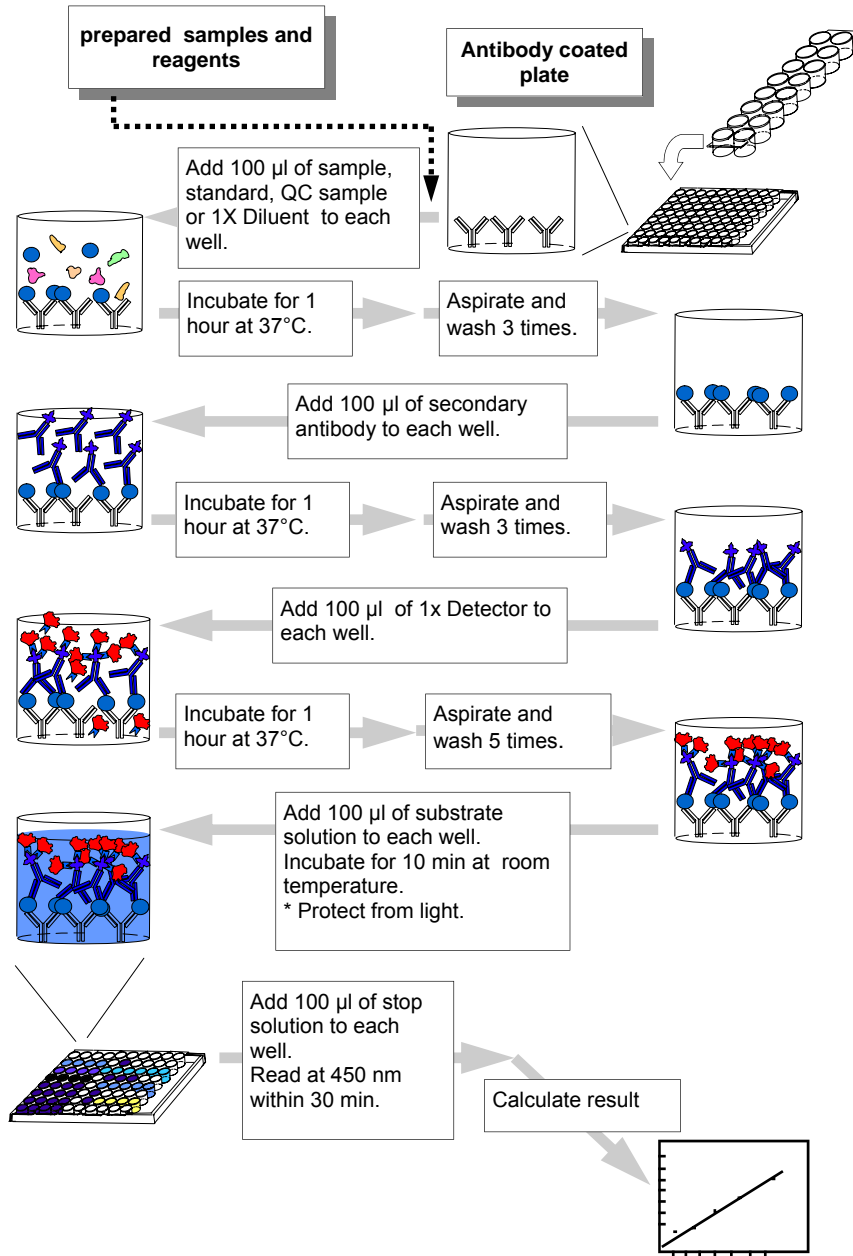
Precision single and multi-channel pipettes
Disposable pipette tips
Microtubes or equivalent for preparing dilutions
Disposable plastic containers for preparing working reagents
Reagent reservoirs
Microwell or microstrip plate reader 450 nm
Deionized water

Sample Collection and Storage

Serum Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

Plasma Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

Flow Chart of Assay Procedure



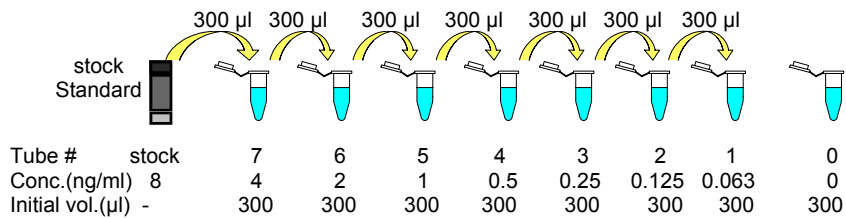
Assay Procedure

1. Preparation of Reagents

- 1) Allow all samples and kit components to equilibrate to room temperature (20-25°C).
- 2) Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use.
It is recommended that Standards and samples be run in duplicate.
- 3) Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
- 4) Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
- 5) Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
- 6) Warm **Substrate Solution** to room temperature before use.
- 7) Prepare working aliquots of the Standard as follows :
When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water to the Standard vial to make a stock concentration of 8 ng/ml. Mix well.

A recommended dilution scheme is as follows :

- A) Label 8 microcentrifuge tubes #0-7. Add 300 μ l of the 1X Diluent to the microcentrifuge tubes # 0-7.
- B) Add 300 μ l of the stock Standard solution to tube # 7 and vortex. This is Standard tube # 7 with a concentration of 4 ng/ml.
- C) Standards # 6 to # 1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube # 0.



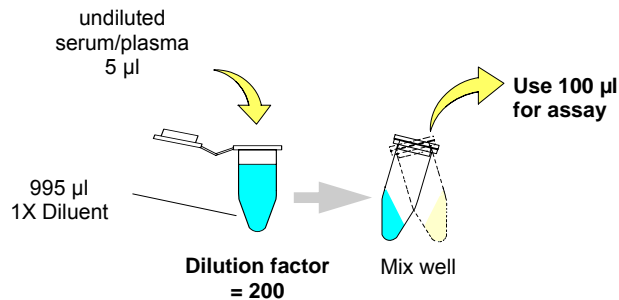
8) Reconstitute QC sample in 1 ml of deionized water. Mix well.

2. Sample Preparation

1) Dilute samples 1:200 with 1X Diluent (example, 5 μ l sample plus 995 μ l 1X Diluent; dilution factor=200) and mix well.

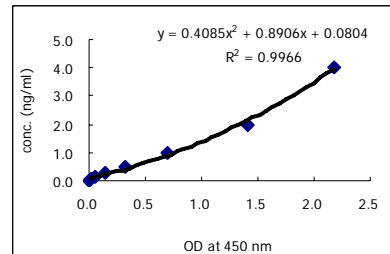
* If samples fall the outside range of assay, a lower or higher dilution may be required.

2) Use 100 μ l of the final diluted sample for ELISA.



3. Experiment procedure

- 1) Remove the appropriate number of microwell strips from the sealed foil pouch.
- 2) Pipette 100 μ l of standard # 0 to # 7, the reconstituted QC sample and diluted serum or plasma into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
- 3) Incubate at 37°C for 1 hour.
- 4) Remove the solution and wash 3 times with 300 μ l of 1X Wash Solution to each well.
- 5) Add 100 μ l Secondary Antibody to each well.
- 6) Incubate at 37°C for 1 hour.
- 7) Remove the solution and wash 3 times with 300 μ l of 1X Wash Solution to each well.
- 8) Add 100 μ l 1X Detector to each well.
- 9) Incubate at 37°C for 1 hour.
- 10) Remove the solution and wash 5 times with 300 μ l of 1X Wash Solution to each well.
- 11) Add 100 μ l of the Substrate Solution to each well.
- 12) Incubate at room temperature for 10 min.
* Protect from light.
- 13) Using the multi-channel pipette, add 100 μ l Stop Solution to each well.
- 14) Read at 450 nm.
- 15) Subtract the absorbance of the blank from the readings for each standard and sample.
- 16) Construct a standard curve by plotting the known concentrations (Y) of standard versus the absorbances (X) of standard. A measurable range is typically shown between 0.063 ng/ml and 4 ng/ml.
- 17) Calculate the progranulin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
- 18) The progranulin concentrations calculated must be multiplied by dilution factor [see **2. Sample Preparation**] to obtain the concentrations of the undiluted samples.



Performance Characteristics

1) Sensitivity : 32 pg/ml

2) Precision

a. Intra-Assay (precision within an assay)

6 samples were tested 8 times to assess intra-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	129.31	4.90	3.79
2	137.48	7.75	5.63
3	128.25	8.89	6.93
4	248.28	15.70	6.32
5	144.99	7.36	5.08
6	95.54	2.88	3.02

b. Inter-Assay (precision between assays)

6 samples were tested 5 times to assess inter-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	133.81	9.52	7.115
2	149.66	8.28	5.532
3	128.45	9.41	7.324
4	220.68	10.396	4.711
5	140.61	9.21	6.549
6	92.23	6.50	7.046

3) Specificity

- a. No cross reaction with mouse and rat sera
- b. Cross Reactivity

Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)
Human progranulin	1	100
Human RBP4	10	N. R.
Human adiponectin	10	N. R.
Human visfatin	10	N. R.
Human leptin	10	N. R.
Human RELM β	10	N. R.
Human AGF	10	N. R.
Human FABP4	10	N. R.
Human TNF- α	10	N. R.
Human IL-33	10	N. R.
Human GPX3	10	N. R.
Human resistin	10	N. R.
Human clusterin	10	N. R.
Human vaspin	10	N. R.
Human PAI1	10	N. R.
Human ANGPTL3	10	N. R.
Mouse visfatin	10	N. R.
Rat visfatin	10	N. R.

4) Recovery

The recovery of progranulin spiked to two different levels in five different human serum samples throughout the range of assay was evaluated.

Sample No.	Average recovery (%)	Range (%)
1	97.16	91-103
2	97.98	93-102
3	97.69	95-101
4	95.41	89-99
5	96.73	91-100

5) Linearity - Effect of Serum Dilution

Three serum samples were pre-treated as described in the protocol, resulting in the final dilution of x200 (labeled in the table below as dilution; 1).

Sample No.	Serum Dilution	Expected (ng/ml)	Observed (ng/ml)	% Of Expected
1	1	156.13	156.13	100
	1/2	78.06	78.79	101
	1/4	39.03	39.78	102
2	1	239.69	239.69	100
	1/2	119.84	111.94	93
	1/4	59.92	57.27	96
3	1	165.84	165.84	100
	1/2	82.92	80.94	98
	1/4	41.46	40.26	97

% of expected = observed / expected x 100%

References

1. Bhandari, V.; Palfree, R. G. E.; Bateman, A. : Isolation and sequence of the granulin precursor cDNA from human bone marrow reveals tandem cysteine-rich granulin domains. *Proc. Nat. Acad. Sci.* 89: 1715-1719, 1992.
2. He, Z.; Bateman, A. : Progranulin gene expression regulates epithelial cell growth and promotes tumor growth in vivo. *Cancer Res.* 59: 3222-3229, 1999.
3. He, Z.; Ong, C. H. P.; Halper, J.; Bateman, A. : Progranulin is a mediator of the wound response. *Nature Med.* 9: 225-229, 2003.

Troubleshooting Guide

Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

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