

ELISA Kit for Measuring Human Progranulin

CircuLex Human Progranulin ELISA Kit

Cat# CY-8101

Intended Use..... 1
Storage..... 1
Introduction..... 2
Principle of the Assay..... 2-3
Materials Provided..... 3
Materials Required but not Provided..... 4
Precautions and Recommendations..... 5
Sample Collection and Storage..... 6
Detailed Protocol..... 7-8
Calculations..... 9
Measurement Range..... 9
Troubleshooting..... 9
Reagent Stability..... 9
Assay Characteristics..... 10-12
Example of Test Results..... 13-16
References..... 17-18

Intended Use

The MBL Research Product **CircuLex Human Progranulin ELISA Kit** is used for the quantitative measurement of human progranulin in serum, cell culture supernatant, and other biological samples, e.g. tear, milk saliva and snivel.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at 4°C.
- Don't expose reagents to excessive light.

Introduction

Progranulin (PGRN) also called granulin/epithelin, proepithelin, prostate cancer cell-derived growth factor, acrogranin, or paraganulin, is a 593aa cysteine-rich protein that is typically secreted in a highly glycosylated 88kDa form (1, 2). PGRN is a growth factor involved in the regulation of multiple processes including cell proliferation, tumorigenesis, wound healing, development and inflammation. PGRN is widely expressed in epithelia, bone marrow, immune cells, solid organs, and the nervous system both during development and in adulthood (3-7).

In 2006, mutations in PGRN were discovered to be a cause of frontotemporal lobar degeneration (FTLD) with ubiquitinated TDP-43-positive inclusions (6, 8-10, 12, 13). More than 70 mutations in PGRN, almost all of which result in null alleles, have been identified in FTLD patients. A few causative missense mutations also result in reduced levels of PGRN (10).

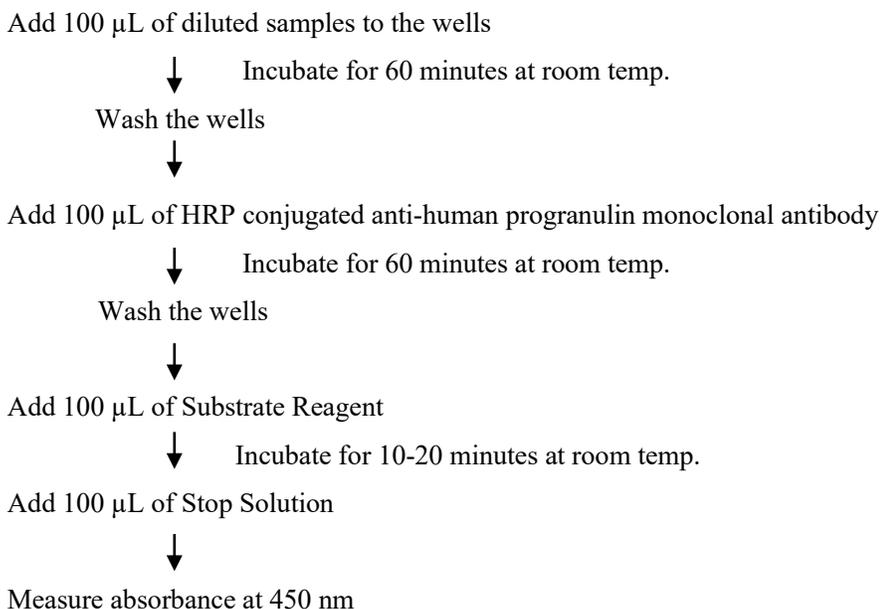
PGRN can be found in adipose tissue, epithelial tissue, gastrointestinal tract, reproductive organs, and so forth (11). Previous studies have demonstrated that increased gene expression of PGRN stimulates cancer cell division, invasion, and against anoikis, promoting tumor formation (2).

It has been shown that PGRN could restrain rheumatoid arthritis by binding directly to tumor necrosis factor receptors (TNFR) and play an anti-inflammatory role in the processes (14). In addition, it was reported that circulating PGRN levels are elevated in patients with type 2 diabetes (15). Moreover, increased plasma PGRN levels are associated with impaired glucose tolerance rather than impaired fasting glucose (16). Although type 2 diabetes is often accompanied by obesity, the respective role of elevation of circulating PGRN levels in obesity and type 2 diabetes remains to be elucidated.

Principle of the Assay

The MBL Research Product **CircuLex Human Progranulin ELISA Kit** employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human progranulin is pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any human progranulin present. After washing away any unbound substances, an HRP conjugated monoclonal antibody specific for human progranulin is added to the wells. Following a wash to remove any unbound antibody HRP conjugate, the remaining conjugate is allowed to react with the substrate H₂O₂-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of human progranulin. A standard curve is constructed by plotting absorbance values versus human progranulin concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

Summary of Procedure



Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for the one 96-well microplate kit.

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with anti-human progranulin monoclonal antibody (KH-3G2) as a capture antibody.

10X Wash Buffer: One bottle containing 100 mL of 10X buffer containing Tween®-20

Dilution Buffer: One bottle containing 50 mL of 1X buffer; use for reconstitution of Human Progranulin Standard and sample dilution. Ready to use.

Human Progranulin Standard: One vial containing X* ng of lyophilized recombinant human progranulin.

***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**

HRP conjugated Detection Antibody: One bottle containing 12 mL of HRP (horseradish peroxidase) conjugated anti-human progranulin monoclonal antibody (KH-3D10). Ready to use.

Substrate Reagent: One bottle containing 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.

Stop Solution: One bottle containing 20 mL of 1 N H₂SO₄. Ready to use.

Materials Required but not Provided

- **Pipettors:** 2-20 μ L, 20-200 μ L and 200-1,000 μ L precision pipettors with disposable tips.
- **Precision repeating pipettor**
- **Orbital microplate shaker**
- **Microcentrifuge and tubes** for sample preparation.
- **Vortex mixer**
- **(Optional) Microplate washer:** Manual washing is possible but not preferable.
- **Plate reader** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **(Optional) Software package facilitating data generation and analysis**
- **500 or 1,000 mL graduated cylinder.**
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**

Precautions and Recommendations

- **Although we suggest to conduct experiments as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user.**
- Allow all the components to come to room temperature before use.
- All microplate strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Use only the microtiter wells provided with the kit.
- Rinse all detergent residues from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents used in this kit contain NaN_3 as preservatives. Care should be taken to avoid direct contact with these reagents.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide.
- Wear gloves and eye protection when handling immunodiagnostic materials and samples of human origin, and these reagents. In case of contact with the Stop Solution and the Substrate Solution, wash skin thoroughly with water and seek medical attention, when necessary.
- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**
- **CAUTION: Sulfuric Acid is a strong acid. Wear disposable gloves and eye protection when handling Stop Solution.**

Sample Collection and Storage

Serum: Use a serum separator tube and allow samples to clot for 60 ± 30 minutes. Centrifuge the samples at 4°C for 10 minutes at 1,000 x g. Remove serum and assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of serum may also be stored at below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Cell culture supernatant: Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles.

Other biological samples: MBL has not tested.

(e.g. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles. Individual users should determine appropriate conditions when using other types of samples.)

Detailed Protocol

The MBL Research Product **CircuLex Human Progranulin ELISA Kit** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since experimental conditions may vary, an aliquot of the Human Progranulin Standard within the kit should be included in each assay as a calibrator. Disposable pipette tips and reagent troughs should be used for all liquid transfers to avoid cross-contamination of reagents or samples.

Preparation of Working Solutions

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready-to-use, with the exception of **10X Wash Buffer** and **Human Progranulin Standard**.

1. Prepare a working solution of Wash Buffer by adding 100 mL of the **10X Wash Buffer** to 900 mL of deionized (distilled) water. Mix well. Store at 4°C for two weeks or -20°C for long-term storage.
2. Reconstitute **Human Progranulin Standard** with **X* µL** of **Dilution Buffer**. The concentration of the human progranulin in vial should be **40 ng/mL**, which is referred to as a **Master Standard** of human progranulin.

***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**

Prepare Standard Solutions as follows:

Use the **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4,000 pg/mL standard (Std.1) serves as the highest standard. The **Dilution Buffer** serves as the zero standard (Blank).

	Volume of Standard	Dilution Buffer	Concentration
Std.1	100 µL of Master Standard (40 ng/mL)	900 µL	4,000 pg/mL
Std.2	300 µL of Std. 1 (4,000 pg/mL)	300 µL	2,000 pg/mL
Std.3	300 µL of Std. 2 (2,000 pg/mL)	300 µL	1,000 pg/mL
Std.4	300 µL of Std. 3 (1,000 pg/mL)	300 µL	500 pg /mL
Std.5	300 µL of Std. 4 (500 pg /mL)	300 µL	250 pg/mL
Std.6	300 µL of Std. 5 (250 pg/mL)	300 µL	125 pg/mL
Std.7	300 µL of Std. 6 (125 pg/mL)	300 µL	62.5 pg /mL
Blank	-	300 µL	0 pg /mL

Note: Do not use a Repeating pipette. Change tips for every dilution. Unused portions of Master Standard should be aliquoted and stored at below -70°C immediately. Avoid multiple freeze and thaw cycles.

Sample Preparation

Dilute samples with **Dilution Buffer**.

- Serum samples may require 50- to 100-fold dilution.
- Tear and saliva samples may require 50- to 100-fold dilution.
- Milk samples may require 200- to 400-fold dilution.

Assay Procedure

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.
2. Dilute samples with **Dilution Buffer**. (See "Sample Preparation" above.)
3. Pipette **100 µL** of **Standard Solutions (Std1-Std7, Blank)** and **diluted samples** in duplicates, into the appropriate wells.
4. Incubate the plate **at room temperature (ca.25°C) for 60 minutes, shaking at ca. 300 rpm on an orbital microplate shaker.**
5. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.
6. Add **100 µL** of **HRP conjugated Detection Antibody** into each well.
7. Incubate the plate **at room temperature (ca.25°C) for 60 minutes, shaking at ca. 300 rpm on an orbital microplate shaker.**
8. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.
9. Add **100 µL** of **Substrate Reagent**. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminum foil is recommended. Return Substrate Reagent to 4°C immediately after the necessary volume is removed
10. Incubate the plate **at room temperature (ca.25°C) for 10-20 minutes, shaking at ca. 300 rpm on an orbital microplate shaker.** The incubation time may be extended up to 30 minutes if the reaction temperature is below 20°C.
11. Add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.
12. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the microplate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Note-1: Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Note-2: Reliable standard curves are obtained when either O.D. values do not exceed 0.25 units for the blank (zero concentration), or 3.0 units for the highest standard concentration.

Note-3: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine human progranulin concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Calculations

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation. To determine the human progranulin concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding human progranulin concentration. If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

1. The dose-response curve of this assay fits best to a sigmoidal 4-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4-parameter logistic function. It is important to make an appropriate mathematical adjustment to accommodate for the dilution factor.
2. Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of calibrators versus log of the known concentration (X) of calibrators, using the 4-parameter function. Alternatively, the logit log function can be used to linearize the calibration curve (i.e. logit of absorbance (Y) is plotted versus log of the known concentration (X) of calibrators).

Measurement Range

The measurement range is 62.5 pg/mL to 4,000 pg/mL. Any sample reading higher than the highest standard should be diluted with Dilution Buffer in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the human progranulin concentration.

Troubleshooting

1. All samples and standards should be assayed in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.
2. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate insufficient washing. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for washer maintenance.
3. Overall low signal may indicate that desiccation of the plate has occurred between the final wash and addition of Substrate Reagent. Do not allow the plate to dry out. Add Substrate Reagent immediately after wash.

Reagent Stability

All of the reagents included in the MBL Research Product **CircuLex Human Progranulin ELISA Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date. Upon receipt, kit reagents should be stored at 4°C, except the reconstituted Human Progranulin Standard must be stored at below -70°C. Coated assay plates should be stored in the original foil bag sealed by the zip lock and containing a desiccant pack.

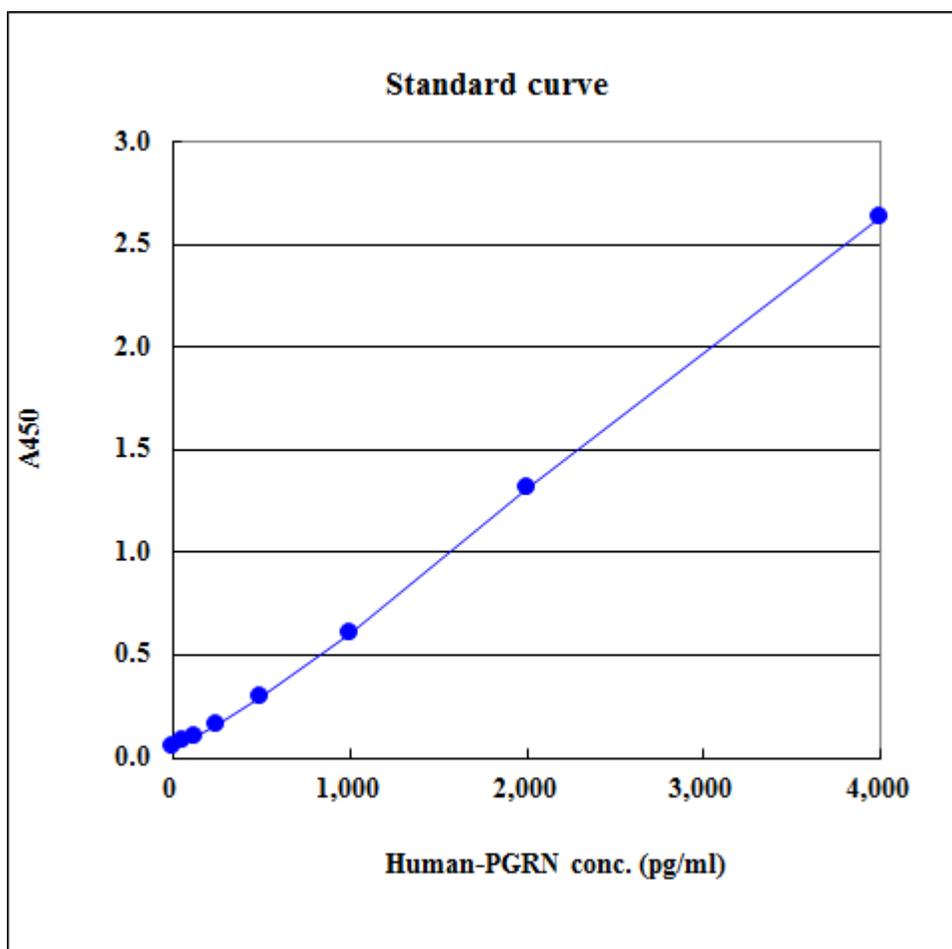
Assay Characteristics

1. Sensitivity

The limit of detection (defined as such a concentration of human progranulin giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 20.4 pg/mL of sample.

* Dilution Buffer was pipetted into blank wells.

Typical Standard Curve



2. Precision

Intra-assay Precision (Precision within an assay)

Three samples* of known concentration were tested six times on one plate to assess intra-assay precision.

- Intra-assay (Within-Run, n=6) CV=4.7-6.1 %

*Sample: human serum

Human progranulin conc. (ng/mL)

No.	Sample 1	Sample 2	Sample 3
1	112.1	98.7	238.0
2	103.5	98.3	228.6
3	108.5	109.7	264.6
4	117.3	108.0	262.9
5	113.2	105.9	235.1
6	117.1	107.9	252.4
max.	117.3	109.7	264.6
min.	103.5	98.3	228.6
mean	112.0	104.8	246.9
SD	5.3	5.0	15.2
CV(%)	4.7	4.8	6.1

Inter-assay Precision (Precision between assays)

Three samples* of known concentration were tested in three separate assays to assess inter-assay precision.

- Inter-assay (Run-to-Run, n=3) CV=2.4-5.4 %

*Sample: human serum

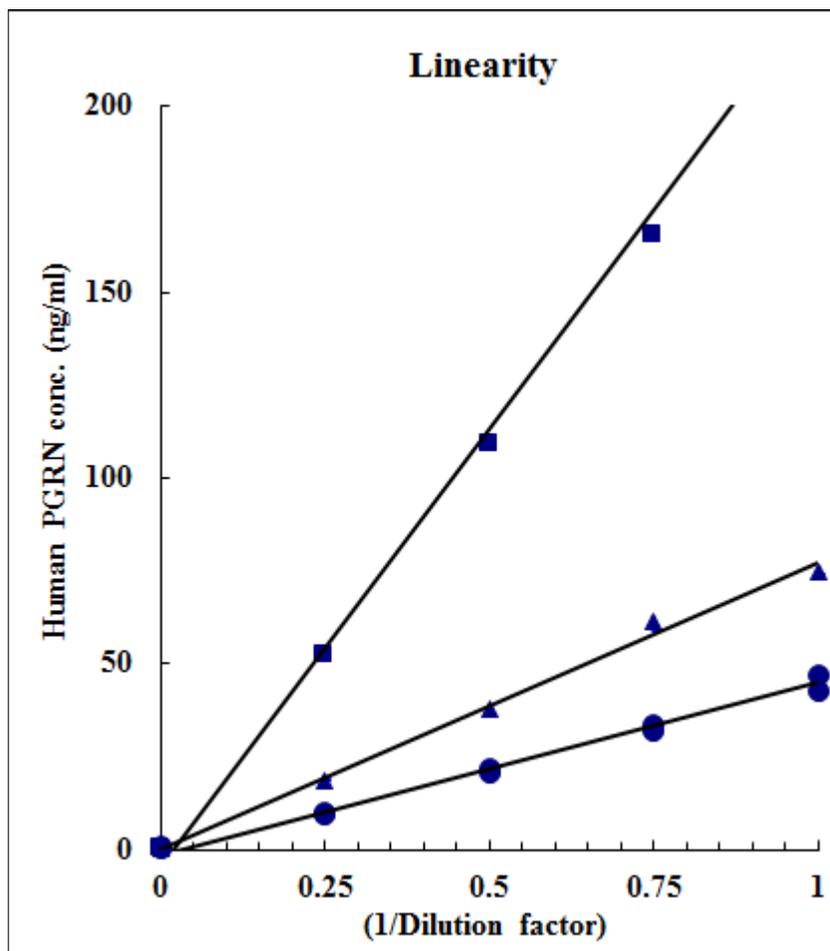
Human progranulin conc. (ng/ml)

Assay	Sample 1	Sample 2	Sample 3
1	47.7	96.1	226.1
2	45.2	87.8	236.3
3	49.7	96.8	227.1
max.	49.7	96.8	236.3
min.	45.2	87.8	226.1
mean	47.6	93.6	229.8
SD	2.3	5.0	5.6
CV(%)	4.7	5.4	2.4

3. Linearity

Three samples* were diluted with Dilution Buffer and assayed after dilution. The neat sample was set to 1. The neat sample was 100-fold diluted as stated in the Assay Procedure. The results was summarized in the figure below.

*Sample: human serum



Example of Test Results

Fig.1 Human progranulin concentration in healthy human sera

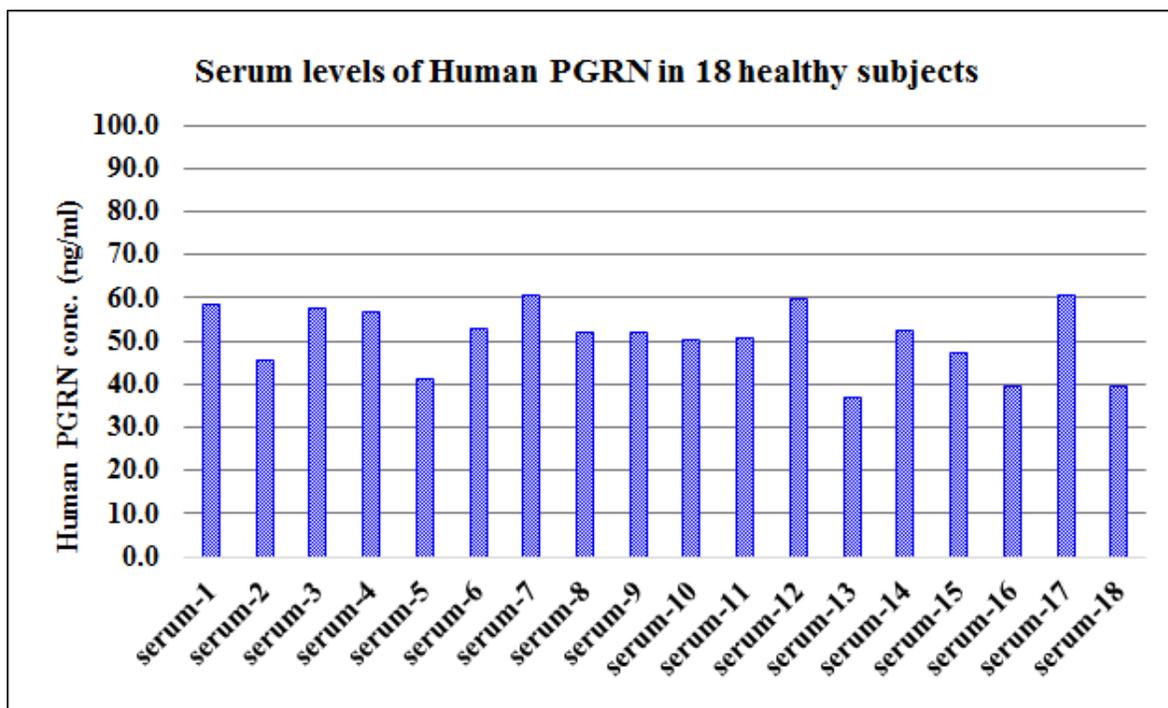


Fig.2 Human progranulin concentration of tear samples

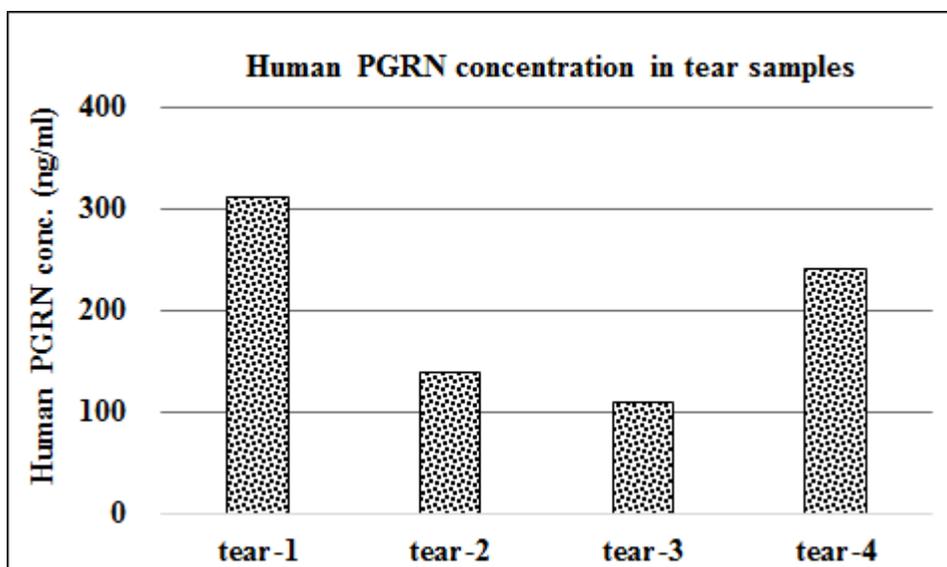


Fig.3 Human progranulin concentration in milk samples

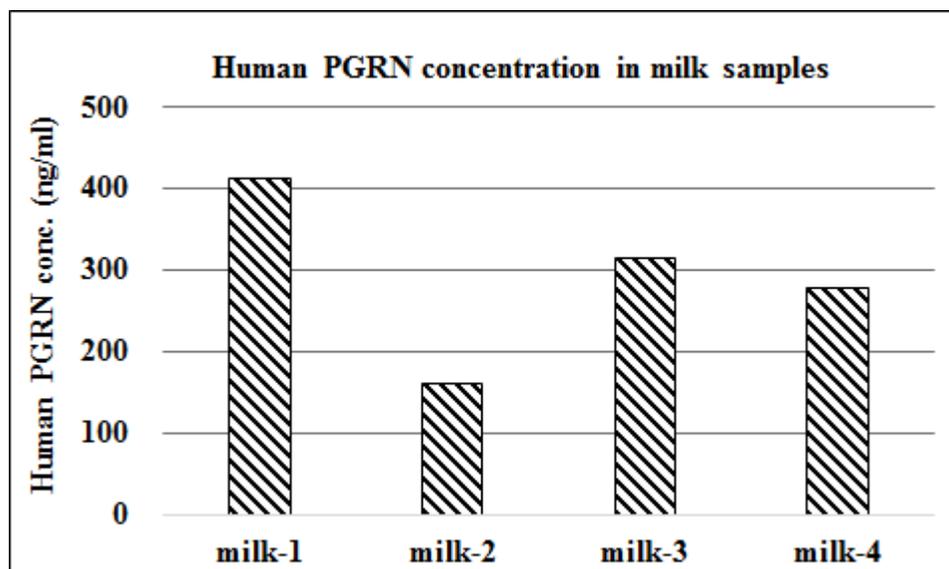


Fig.4 Human progranulin concentration in saliva samples

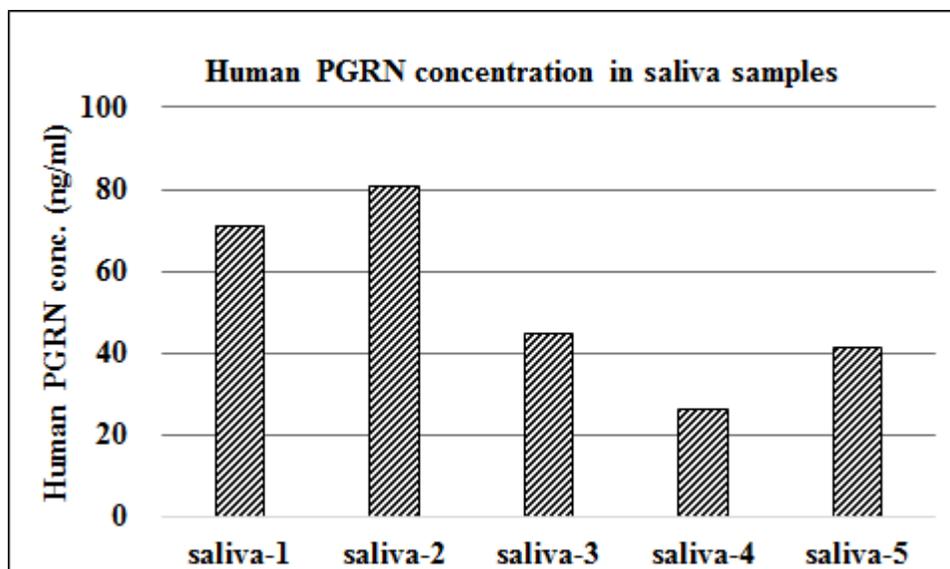


Fig.5 Human progranulin concentration in snivel samples

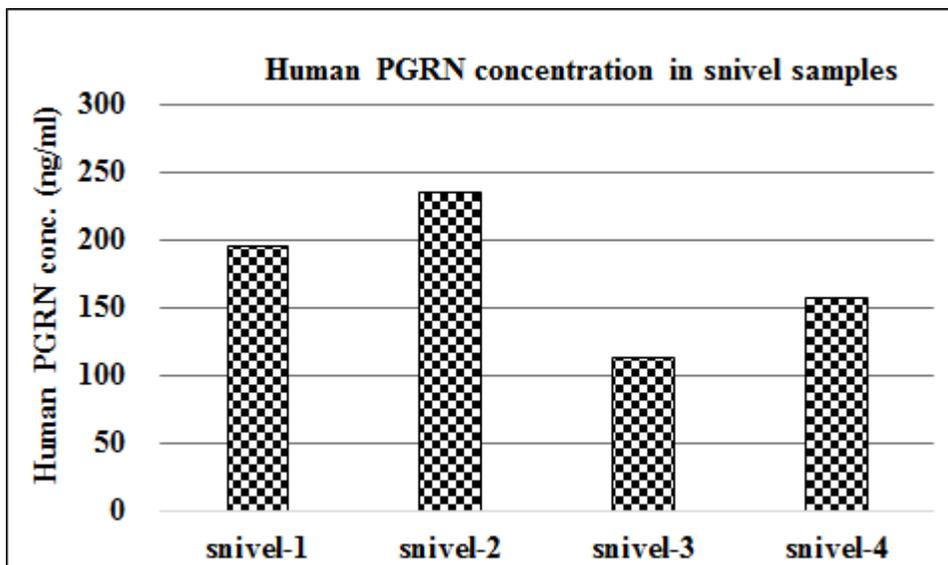


Fig.6 Human progranulin concentration in culture supernatants of cell lines

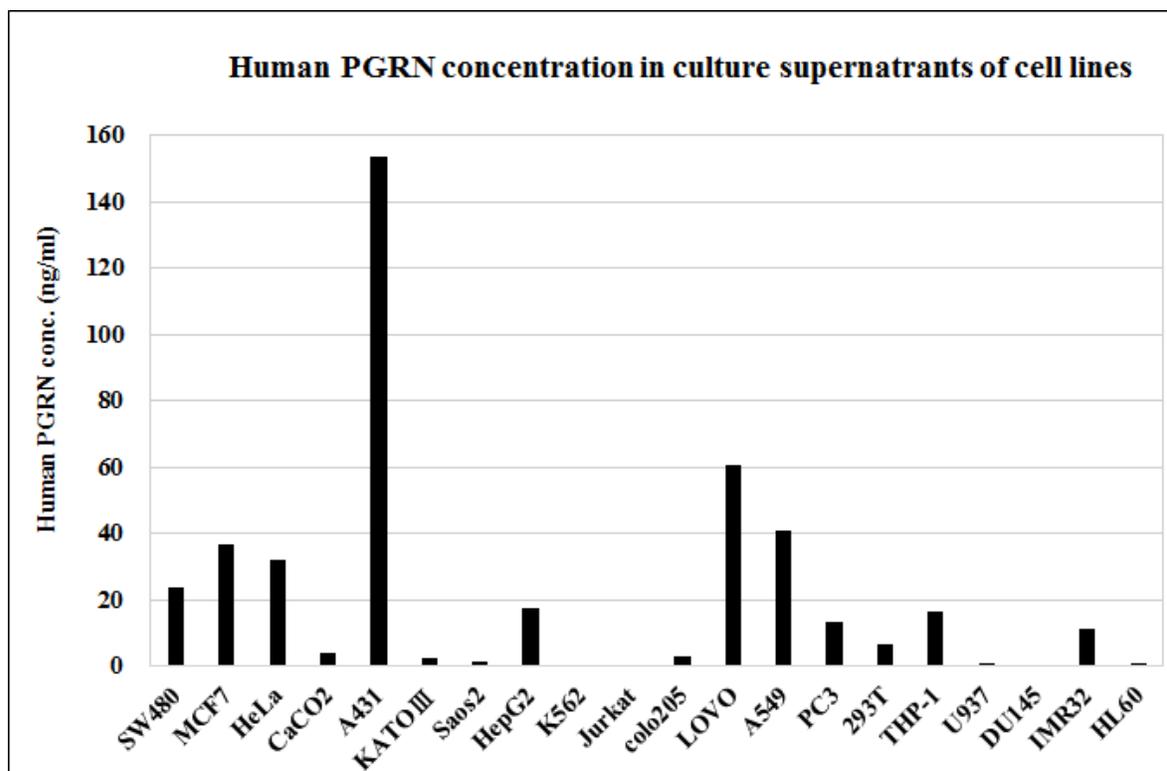
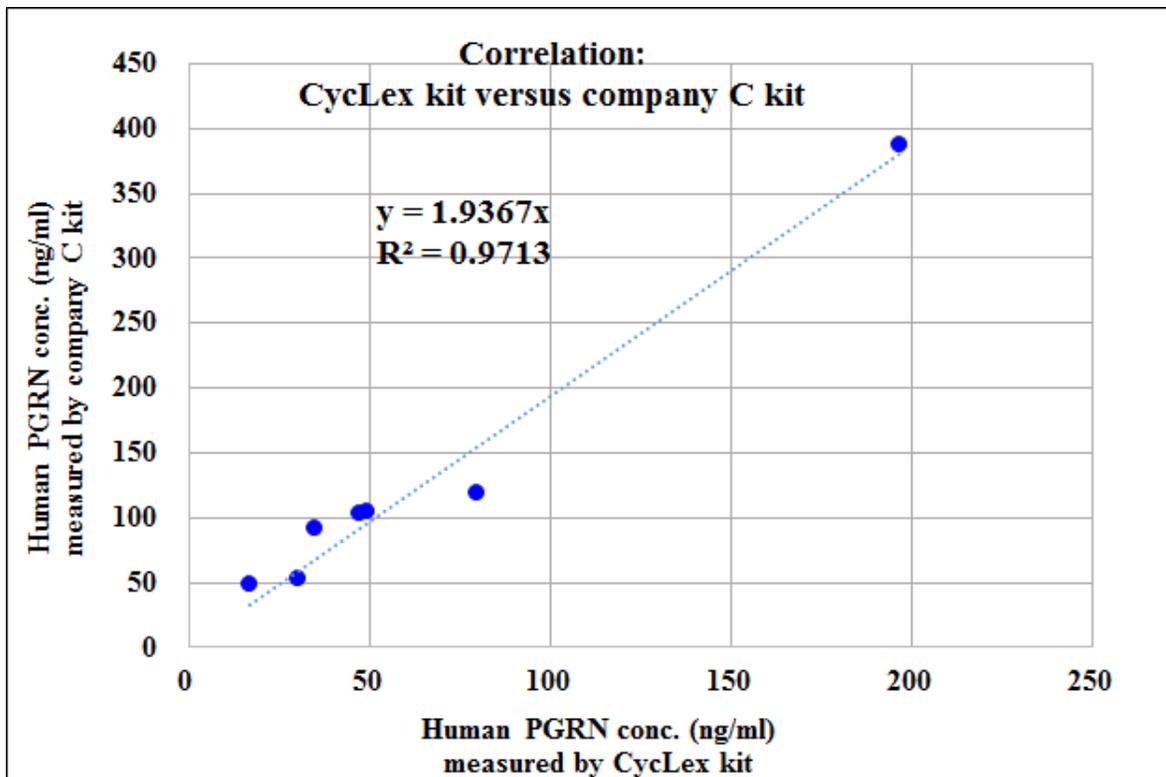


Fig.7 Correlation between human progranulin concentrations in cell culture supernatants measured by ELISA kit of MBL and company C.



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