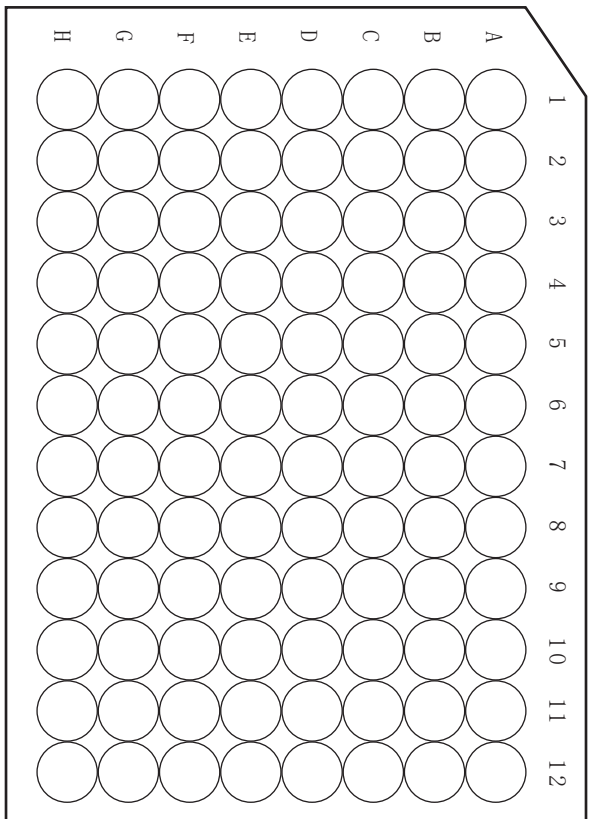

Human GRN Immunoassay

Catalog Number: SEKH-0194

For the quantitative determination of Human GRN concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of human GRN in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	93	98
	Range(%)	85—102	91—104
1:4	Average% of Expected	95	99
	Range(%)	88—106	91—105
1:8	Average% of Expected	97	85
	Range(%)	89-108	76-94
1:16	Average% of Expected	92	94
	Range(%)	84-101	85-107

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 31.25pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Human GRN. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant Human	Recombinant mouse	Recombinant rat
	GRN	

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of Human GRN spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of Human GRN in two matrices

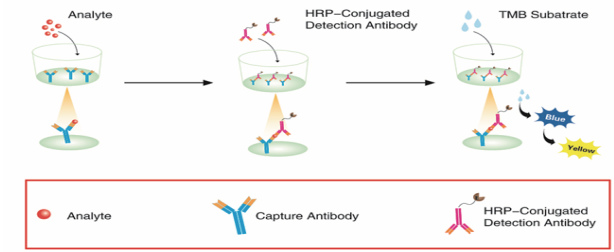
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	92	81—98
Cell culture supernatants	94	86—104

BACKGROUND

GRN (Granulin Precursor, also known as GEP; GP88; PEPI; PGRN; CLN11; PCDGF), located on 17q21.31, is a Protein Coding gene. The gene produces a 63544 Da protein composed of 593 amino acids. The protein contains a 56-residue GrnA sequence, as well as 6 other cysteine-rich granulin-like domains, including GrnB, GrnC, and GrnD, which had previously been known only from N-terminal sequences. Diseases such as Ceroid Lipofuscinosis, Neuronal, 11 and Frontotemporal Lobar Degeneration With Tdp43 Inclusions, Grn-Related are associated with GRN.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for GRN has been pre-coated onto a microplate. Standards and samples are pipetted into the wells; any GRN present is captured by the coated antibody after incubation. After washing away any unbound substances, a HRP-conjugate antibody specific for GRN is added to detect the captured GRN protein in the sample. After extensive washing, a tetramethyl-benzidine (TMB) reagent is added to the wells for signal development. Solution containing sulfuric acid is used to stop color development. The color intensity, proportional to the quantity of bound protein, is then measurable at 450nm.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

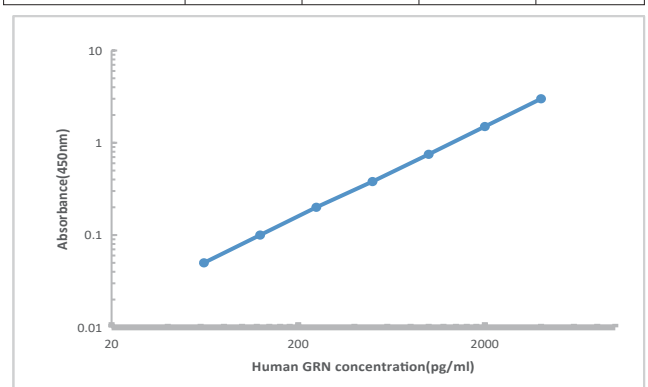
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the Human GRN ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.077	0.073	0.075	--
62.50	0.138	0.206	0.172	0.097
125.0	0.202	0.299	0.250	0.176
250	0.305	0.445	0.375	0.300
500	0.507	0.706	0.607	0.532
1000	0.888	1.147	1.017	0.943
2000	1.599	1.867	1.733	1.658
4000	2.834	3.042	2.938	2.863



Representative standard curve for Human GRN ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of HRP-Conjugate anti- human GRN antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 5-30 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the Human GRN concentrations versus the log of the O.D. and the best fit line can be

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate-antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8°C**
Standard - lyophilized,4000pg/ml upon reconstitution	2 vials	Store at 2-8°C** for six months
HRP Congugated Antibody（100×） - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard /sample Diluent - 16ml/vial	1bottle	Store at 2-8°C** for six months
HRP Congugated Diluent - 16ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

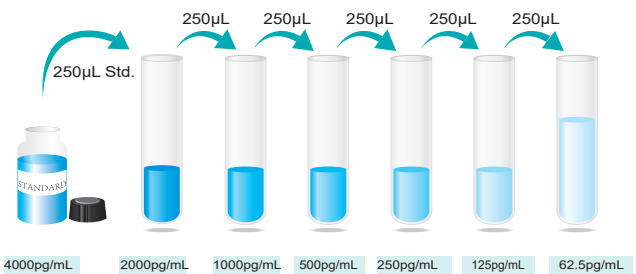
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard\Sample** - Reconstitute the Standard with 0.5mL of Standard/Sample Diluent. This reconstitution produces a stock solution

of 4000pg/mL.Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250uL of Standard/Sample Diluent into 2000pg/ml tube and the remaining tubes. Use the stock solution of 4000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 4000pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of GRN standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of HRP-Congugated Antibody(100×):Make a 1:100 dilution in Reagent Diluent. If the entire 96-well plate is used, add 100uL of HRP Conjugate to 10mL of HRP-Congugated Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE**
***The working solution should be used within one day after dilution.**