HUMAN ACROGRANIN/ PROGRANULIN (PGRN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ACROGRANIN/PROGRANULIN (PGRN) CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA.

A BIOMARKER FOR ALZHEIMER'S DISEASE & TUMOR

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Purchase Information:

ELISA NAME	HUMAN ACROGRANIN/PROGRANULIN ELISA
Catalog No.	SK00313-06
Formulation	96 T
Lot No.	
Standard range	0.32-200 ng/mL
Sensitivity	1.6 ng/mL
Sample Volume	50 µl
Sample Dilution	5 (*Optimal dilutions should be determined by each laboratory for each application*)
Sample Type	Serum, EDTA Plasma, cell culture
Specificity	Human progranulin only
Intra-assay Precision	6-8%
Inter-assay Precision	10-12%
Storage	2 °C-8 °C

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INTRODUCTION

Human progranulin immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human progranulin in cell culture supernates, serum, and plasma. It contains recombinant human progranulin and antibodies raised against this protein. It has been shown to accurately quantitie recombinant human progranulin. Results obtained with naturally occurring progranulin samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the Immunoassay kit can be used to determine relative mass values for natural human progranulin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for progranulin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any progranulin present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for progranulin is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of progranulin bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with polyclonal IgG against rabbit IgG	RM01	1 plate
Progranulin Standard – 200 ng/vial of recombinant human progranulin in a buffered protein base with preservatives; lyophilized.	313-06-02	1 vial
Antibody Concentrate– 350 µL / vial, 10-fold concentrated of Biotinylated polyclonal antibody against progranulin with preservatives; lyophilized.	313-06-03	1 vial
Biotin Solution-350 µL / vial, 10-fold concentrated of human Progranulin Biotinylated against with preservatives; lyophilized.	313-06-01	1 vial
Positive Control- one of recombinant human progranulin, lyophilized	313-06-04	1 vial
Streptavidin-HRP Conjugate - 120 ul/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
Dilution Buffer- 60mL/vial of buffered protein based solution with preservatives	DB18	1 vial
HRP Diluent Solution- 12ml/vial of buffered protein based solution with preservatives. Ready to ues	DB06C	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution - 11 mL / vial of TMB substrate solution	TMB01	1 vial

Stop Solution - 11 mL /vial of 0.5N HCl	S-STOP	1 vial
Plate Sealer	EAPS	1 piece

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate as well as Dilution Buffer should be stored at -20°C or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard , Antibody Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and 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 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and 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 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

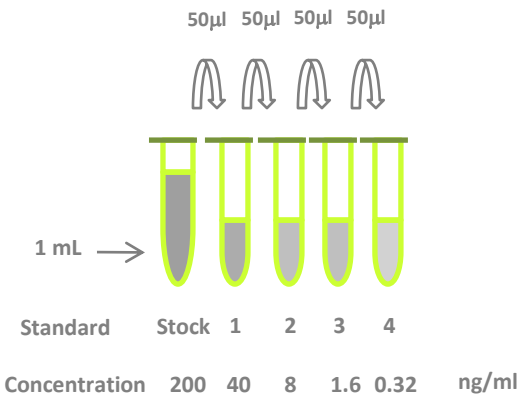
SAMPLE PREPARATION

Serum and plasma samples require a 5-fold dilution. A suggested 5-fold dilution is 30 µL sample + 120 µL Dilution Buffer.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.
Progranulin Standard - Refer to vial label for reconstitution volume. Reconstitute the **Progranulin Standard** with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 200 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 50 µL of the appropriate Dilution Buffer into tubes #1 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 ng/mL standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1000 µl	200 ng/ml
# 1	50 µl of stock	200 µl	40 ng/ml
# 2	50 µl of 1	200 µl	8 ng/ml
# 3	50 µl of 2	200 µl	1.6 ng/ml
# 4	50 µl of 3	200 µl	0.32 ng/ml



Antibody- Reconstitute the **Antibody** with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 3.15 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer 350 µl of 10-fold concentrated stock solution to prepare working solution.

Biotin Solution- Reconstitute it with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 3.15 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer 350 µl of 10-fold concentrated stock solution to prepare working solution.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into the 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution.

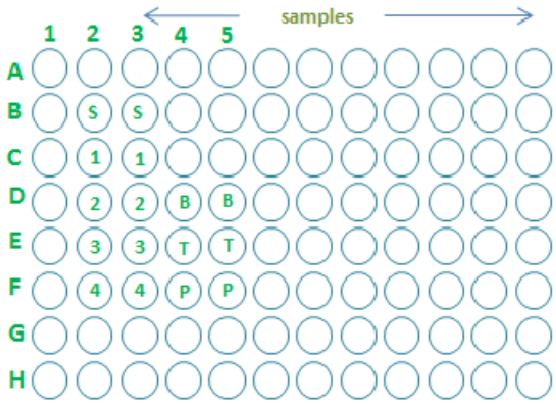
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic bag containing the desiccant pack, reseal.
3. Leave well D4 and D5 as Blank. **DO NOT add Antibody or Biotin Solution into Blank wells.**
4. Add 50 µL of Dilution Buffer to Total binding wells (E4, E5). Add 50 µL of Standard (B2, B3 to F2, F3). Add 50 µL of Positive Control into well F4 and F5. Add 50 µL of samples into appropriate wells. A plate layout is provided to record standards and samples assayed.
5. Add 25 µL of Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. **Note: DO NOT ASPIRATE OR WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.**
6. Add 25 µL of Biotin Solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. 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.
8. Add 100 µL of **Streptavidin-HRP Conjugate** working solution to all wells (including Blank wells). Incubate for 45 min on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 7.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-10 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create 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. The data may be linearized by plotting the log of the progranulin concentrations versus the log of the O.D. and the best fit line can be 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.



TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0.052
200	0.100
40	0.255
8	0.758
1.6	1.227
0.32	1.333
Total Binding	1.440

- *** Lot No.:**
- **Positive Control: 4.99 – 9.27 ng/ml**

LINEARITY

To assess the linearity of the assay, pooled research human serum samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1 X	36.689	36.689	100
5 X	7.751	38.755	105.63
10 X	4.746	47.46	129.36

To assess the linearity of the assay, pooled research human EDTA plasma samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1 X	55.839	55.839	100
5 X	10.459	52.295	93.65
10 X	5.951	59.51	106.57

CALIBRATION

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant human Acrogranin/progranulin.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of progranulin was 1.6 ng/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human progranulin. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rh progranulin control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEIN	CROSS-REACTIVITY (%)
Human Progranulin	100
Rat Progranulin	100
Human BDNF	0
Human CTGF	0
Human SDF-1α	0
Human NRG4	0
Human FABP7	0

REFERENCES

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SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 50µl of standard, samples, positive control to each well. Add 25 µl of Antibody solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
DO NOT ASPIRATE OR WASH PLATE. Add 25 µl Biotin Solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptatvin HRP conjugate to all wells. Incubate 45 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 3-10min on the bench top. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min