Quantikine® ELISA

Human Progranulin Immunoassay

Catalog Number DPGRN0

For the quantitative determination of human Progranulin concentrations in cell culture supernates, serum, plasma, saliva, urine, and human milk.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Progranulin, also known as acrogranin, PC cell-derived growth factor (PCDGF), and epithelin/granulin precursor, is a ubiquitously expressed 88 kDa secreted glycoprotein (1-3). Structurally, it belongs to none of the well-established growth factor families (4). Human progranulin is 593 amino acids (aa) in length and contains a 17 aa signal sequence and 5 potential sites for N-linked glycosylation (SwissProt #: P28799). It has a highly repetitive organization, containing seven tandem copies of a 55-57 aa consensus motif that contains 12 conserved cysteine residues: $VXCX_{5-6}CX_5CCX_8CCX_6CCXDX_2HCCPX_4CX_{5-6}CX_2$ (1). There are two isoforms for human progranulin. Isoform 2 has a deletion corresponding to aa 377-531 in isoform 1.

Progranulin is secreted in its intact form (2, 4), but in peripheral tissues, extracellular proteases, such as elastase, were shown to be able to cleave progranulin into its constituent peptides made from the seven tandem repeats (granulins A-F and paragranulin), which probably have separate functions (5-7). Human progranulin shares 75% aa sequence identity with mouse and rat progranulin.

Progranulin is involved in the regulation of cellular proliferation, as well as differentiation, development, and pathological processes (4). It has been isolated as a differentially expressed gene from mesothelial differentiation (8), macrophage development (9), synovium of rheumatoid arthritis and osteoarthritis (10), sexual differentiation of the brain (11), and was also shown to be a mediator of cartilage proliferation, wound response, and tissue repair (4, 7, 12). High levels of progranulin expression have been found to be associated with several human cancers and are believed to contribute to tumorigenesis in breast cancer, clear cell renal carcinoma, invasive ovarian carcinoma, glioblastoma, adipocyte teratoma, and multiple myeloma (4, 12-18).

Recently, there has been increased interest in progranulin, particularly in its role in the central nervous system and in neurodegenerative diseases. Little is known about the role of progranulin in the central nervous system (5). Progranulin is widely expressed during early neural development (5, 19). Later on, its expression becomes restricted to defined neuronal populations, such as cortical and hippocampal pyramidal neurons and Purkinje cells (20). Progranulin is upregulated in activated microglial cells but not in astrocytes or oligodendrocytes (5, 21-24). Recent research shows that progranulin is a neurotrophic factor with activities that may be involved in the development of the nervous system and in neurodegeneration (5). Null mutations in the progranulin gene are also a common cause of autosomal dominant tau-negative frontotemporal lobe dementia (25). Increased expression of progranulin is seen in activated microglia in other neurodegenerative diseases including Creutzfeldt-Jakob disease, motor neuron disease, and Alzheimer's disease (22).

The Quantikine® Human Progranulin immunoassay is a 4.5 hour solid phase ELISA designed to measure human Progranulin in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains NS0-expressed recombinant human Progranulin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural Progranulin showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human Progranulin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human 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, an enzyme-linked monoclonal antibody specific for human Progranulin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, 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.
- Samples, controls, and standards must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Progranulin Microplate	893697	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Progranulin.	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.*
Human Progranulin Conjugate	893698	21 mL of a monoclonal antibody specific for human Progranulin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Progranulin Standard	893699	Recombinant human Progranulin in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-5	895608	11 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD6-23	895275	21 mL of animal serum with preservative. *Use diluted 1:2 for serum/plasma/urine/human milk samples. Use diluted 1:4 for cell culture supernate/saliva samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human Progranulin Controls (optional; R&D Systems®, Catalog # QC147).

PRECAUTIONS

High concentrations of Progranulin are found in saliva. Take necessary precautions to protect kit reagents.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 at room temperature 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 or heparin 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.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer and assay immediately, or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva values are decreased when a Salivette® or other collection device is used. When stored at 2-8 °C, saliva sample values increase over time.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and centrifuge twice more for a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD6-23 (diluted 1:2)*.

Urine samples require a 2-fold dilution. A suggested 2-fold dilution is 200 µL of sample + 200 µL of Calibrator Diluent RD6-23 (diluted 1:2)*.

Saliva samples require a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD6-23 (diluted 1:4)*.

Human milk samples require a 6-fold dilution. A suggested 6-fold dilution is 50 µL of sample + 250 µL of Calibrator Diluent RD6-23 (diluted 1:2)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of Progranulin are found in saliva. Take necessary precautions to protect kit reagents.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

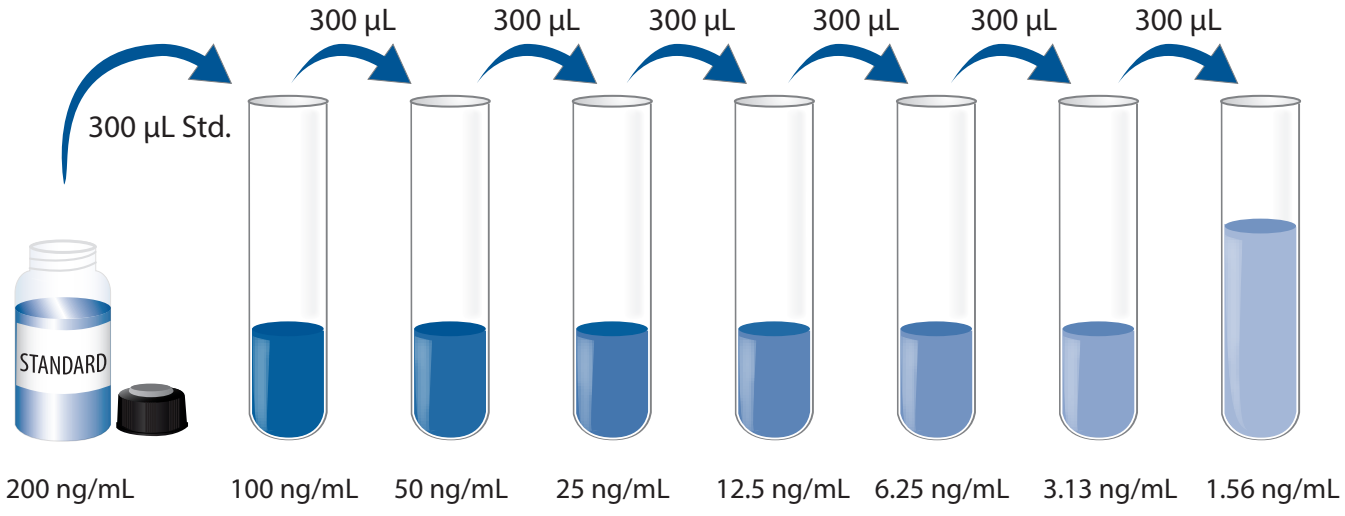
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD6-23 (diluted 1:2) - **For serum, plasma, urine, or human milk samples**. Add 10 mL of Calibrator Diluent RD6-23 to 10 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6-23 (diluted 1:2).

Calibrator Diluent RD6-23 (diluted 1:4) - **For cell culture supernate or saliva samples.** Add 5 mL of Calibrator Diluent RD6-23 to 15 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6-23 (diluted 1:4).

Human Progranulin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Progranulin Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 300 µL of Calibrator Diluent RD6-23 (diluted 1:2) (*for serum, plasma, urine, and human milk samples*) or Calibrator Diluent RD6-23 (diluted 1:4) (*for cell culture supernate and saliva samples*) into seven tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 100 ng/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

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

Note: *High concentrations of Progranulin are found in saliva. Take necessary precautions to protect kit reagents.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-5 to each well. *Assay Diluent RD1-5 may contain a precipitate. Mix well before and during use.*
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.

   Note: *Standard, control, and samples must be pipetted within 15 minutes.*
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 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.
6. Add 200 µL of Human Progranulin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

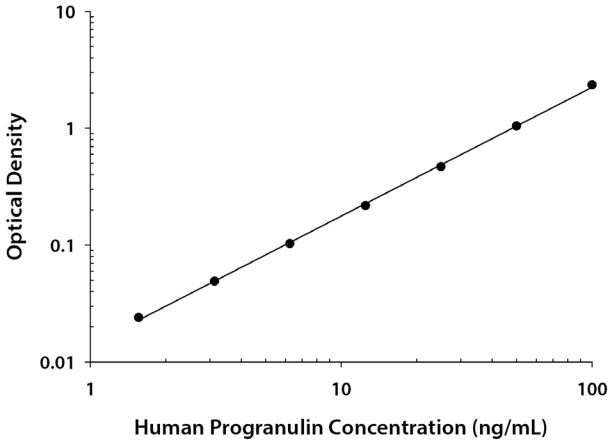
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human Progranulin concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

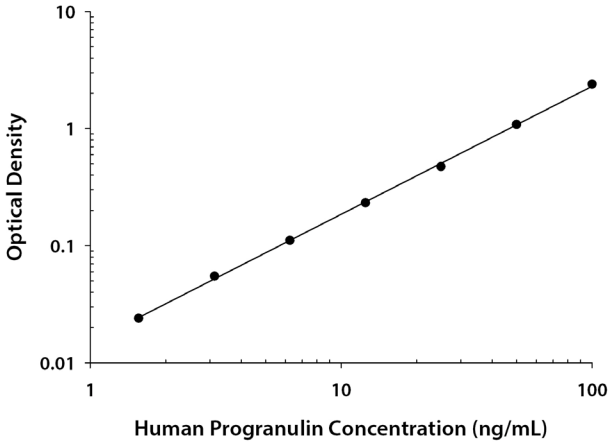
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

SERUM/PLASMA/URINE HUMAN MILK ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.011 0.012	0.012	—
1.56	0.035 0.036	0.036	0.024
3.13	0.060 0.062	0.061	0.049
6.25	0.114 0.115	0.115	0.103
12.5	0.216 0.244	0.230	0.218
25	0.479 0.482	0.481	0.469
50	1.020 1.090	1.055	1.043
100	2.283 2.442	2.363	2.351

CELL CULTURE SUPERNATE/SALIVA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.011 0.012	0.012	—
1.56	0.036 0.036	0.036	0.024
3.13	0.065 0.069	0.067	0.055
6.25	0.123 0.123	0.123	0.111
12.5	0.243 0.244	0.244	0.232
25	0.464 0.505	0.485	0.473
50	1.079 1.111	1.095	1.083
100	2.340 2.465	2.403	2.391

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/SALIVA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	13.5	32.7	64.8	14.4	33.1	64.4
Standard deviation	0.7	1.9	4.1	1.0	2.3	4.4
CV (%)	5.2	5.8	6.3	6.9	6.9	6.8

SERUM/PLASMA/URINE/HUMAN MILK ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	15.5	36.6	70.8	15.7	35.8	69.1
Standard deviation	0.9	1.7	3.1	1.3	2.9	5.1
CV (%)	5.8	4.6	4.4	8.3	8.1	7.4

RECOVERY

The recovery of human Progranulin spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	107	103-112%
Serum* (n=4)	104	90-115%
EDTA plasma* (n=4)	102	95-110%
Heparin plasma* (n=4)	100	92-108%
Urine* (n=4)	109	99-116%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Progranulin were serially diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Saliva* (n=4)	Urine* (n=4)	Human milk* (n=4)
1:2	Average % of Expected	97	103	103	103	97	97	100
	Range (%)	91-106	101-106	100-109	95-107	92-102	96-98	97-104
1:4	Average % of Expected	101	105	103	102	95	97	102
	Range (%)	92-111	102-111	99-106	94-109	91-99	94-102	97-109
1:8	Average % of Expected	100	106	104	103	98	96	101
	Range (%)	97-106	103-111	98-111	92-113	92-104	93-101	94-107
1:16	Average % of Expected	100	107	104	105	100	99	106
	Range (%)	95-110	105-111	93-109	92-113	96-111	97-100	100-115

*Samples were diluted prior to assay as described in the Sample Preparation section.

SENSITIVITY

One hundred thirty-five assays were evaluated and the minimum detectable dose (MDD) of human Progranulin ranged from 0.05-0.54 ng/mL. The mean MDD was 0.17 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human Progranulin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human Progranulin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	51.6	33.0-80.8	11.5
EDTA plasma (n=36)	44.2	27.4-67.2	9.9
Heparin plasma (n=36)	45.1	28.3-67.6	9.5
Saliva (n=10)	119	48.4-304	74
Urine* (n=10)	9.51	3.34-18.1	——
Human Milk (n=11)	143	48.3-406	109

*Two samples measured below the lowest standard, 1.56 ng/mL, and are not factored into the data above.

SAMPLE VALUES *CONTINUED*

Cell Culture Supernates:
Human peripheral blood leukocytes were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human Progranulin.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	ND	3.16
Stimulated	ND	6.40

ND=Non-detactable

MCF-7 human breast cancer cells were cultured in F-12/DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for human Progranulin, and measured 5.47 ng/mL.

MCF 10A human breast epithelial cells were cultured in 50% Ham's F-12 media and 50% DMEM supplemented with 5% equine serum, 100 ng/mL cholera enterotoxin, 10 µg/mL insulin, 0.5 µg/mL hydrocortisol, and 20 ng/mL of recombinant human EGF. An aliquot of the cell culture supernate was removed, assayed for human Progranulin, and measured 12.3 ng/mL.

A549 human lung carcinoma cells were cultured in Kaighn's F-12 media supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for levels of human Progranulin, and measured 3.12 ng/mL.

ME-180 human cervical epithelial carcinoma cells were cultured in McCoy's 5a media supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human Progranulin, and measured 15.4 ng/mL.

PC-3 human prostate cancer cells were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human Progranulin, and measured 36.3 ng/mL.

OVCAR-3 human ovarian carcinoma cells were cultured in RPMI supplemented with 20% fetal bovine serum, 10 µg/mL bovine insulin, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, and 1.5 g/L sodium bicarbonate until confluent. An aliquot of the cell culture supernate was removed, assayed for human Progranulin, and measured 11.0 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Progranulin.

The factors listed below were prepared at 1000 ng/mL in the appropriate calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 1000 ng/mL in a mid-range recombinant human Progranulin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

EG-VEGF
MAPK-1
MAPK-3
PDGF-AA
PDGF-AB
PDGF-BB
PlGF
SLPI
TNF-α
TNF RI
TNF RII
$VEGF_{121}$
$VEGF_{165}$
$VEGF_{206}$
$VEGF\text{-}B_{167}$
VEGF-C
VEGF-D

Recombinant mouse:

EG-VEGF
PlGF-2
Progranulin
SLPI
TNF-α
TNF RI
TNF RII
$VEGF_{120}$
$VEGF\text{-}B_{186}$
VEGF-D

Recombinant canine:

TNF RI

Natural proteins:

human PDGF

Trypsin- and Elastase-cleaved Progranulin show reduced recognition in this assay demonstrating specificity for Progranulin.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.