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R&D SYSTEMS

Quantikine™ QuicKit™ ELISA

Human Granulysin Immunoassay

Catalog Number QK3138
SK3138
PK3138

For the quantitative determination of human Granulysin concentrations in cell culture supernates, cell lysates, serum, and plasma.

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

TABLE OF CONTENTS

SECTION PAGE

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INTRODUCTION

Granulysin (formerly NKG5 or Lymphokine LAG2) is a member of the saposin-like protein (SAPLIP) family of membrane disrupting proteins (1). Granulysin is expressed in granules of natural killer and activated cytotoxic T cells. It exhibits cytolytic activity against intracellular or extracellular microbes and also tumors, either alone or in synergy with perforin (2). Human granulysin has structural similarity and 30 40% aa identity to granulysins and NKlysins of other mammals such as bovine, porcine and canine; similar peptides in rodents have not been identified (1). The 15 kDa unglycosylated protein contains five helical domains; helix 2 and 3 contain 9 arginines and one cysteine critical for activity. Peptides of either helix 2 or 3 will lyse bacteria, while helix 3 is needed to lyse tumor targets (3, 4). One isoform designated 519 uses a different start codon, has no signal peptide sequence and is poorly expressed (5). A posttranslationally processed 9 kDa form is present in acidified granules and is less lytic than the 15 kDa form at granule pH (6). IL15 is necessary and sufficient for granulysin upregulation in CD8 T cells (2). Nanomolar granulysin promotes chemotaxis and increases production of chemokines by monocytic cells, while micromolar local concentrations are needed for lysis (7). Experimental evidence supports the following mechanism for activity against intracellular pathogens (8). First, granulysin forms clusters in lipid rafts due to interaction of positive charges in helices 23 with acidic sphingolipids. After endocytosis, early endosomes fuse with phagosomes, probably regulated by small GTPase rab5. Granulysin binds microbial membranes through charge interactions and disrupts them, probably via scissoring actions of granulysin molecules (9, 10).

The Quantikine™ QuicKit™ Human Granulysin Immunoassay is a one-step, 80-minute solid phase ELISA designed to measure human Granulysin levels in cell culture supernates, serum, plasma, and lysates. It contains recombinant human Granulysin and antibodies raised against the recombinant protein. Results obtained for naturally occurring human Granulysin showed linear curves that were parallel to the standard curves obtained using the recombinant QuicKit standards. These results indicate that this kit can be used to determine relative mass values for natural human Granulysin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled polyclonal capture antibody and an enzyme-linked monoclonal detection antibody, specific for human Granulysin. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of Granulysin bound. 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 from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the calibrator diluent and repeat the assay.
- Any variation in 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™ QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step 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 #	CATALOG # QK3138	CATALOG # SK3138	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
QuicKit™ Coated Microplate	899063	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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 Granulysin Standard	899616	2 vials	12 vials	Recombinant human Granulysin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for the reconstitution volume.*	Use a freshly reconstituted standard for each assay. Discard after use.
Human Granulysin Capture Ab Concentrate	899614	1 vial	6 vials	Lyophilized tagged polyclonal antibody specific for human Granulysin.	May be stored for up to 1 month at 2-8 °C.*
Human Granulysin Detection Ab Concentrate	899615	1 vial	6 vials	400 µL of a monoclonal antibody specific for human Granulysin conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-111	895976	1 vial	6 vials	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-74	896167	1 vial	6 vials	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
TMB ELISA Substrate	642736	1 vial	6 vials	12 mL of a TMB ELISA substrate.	
ELISA Stop Solution	642827	1 vial	6 vials	12 mL of an acid solution.	
Plate Sealers	N/A	4 strips	8 strips	Adhesive strips.	

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

QK3138 contains sufficient materials to run an ELISA on one 96 well plate.
SK3138 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PK3138). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak has enough reagents to assay 50 microplates (96 wells/plate). Although the inserts are the same as those for the single kit inserts, there are minor differences related to the number of reagents and their container sizes.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase* (R&D Systems™, Catalog # WA126).

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
QuicKit™ Coated Microplate	899063	50 plates
Human Granulysin Standard	899616	25 vials
Human Granulysin Capture Ab Concentrate	899614	50 vials
Human Granulysin Detection Ab Concentrate	899615	50 vials
Assay Diluent RD1-111	895976	50 vials
Calibrator Diluent RD5-74	896167	50 vials
Wash Buffer Concentrate	895126	9 bottles
TMB ELISA Substrate	642736	50 vials
ELISA Stop Solution	642827	50 vials
Plate Sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

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
- 500 mL graduated cylinder
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- **Polypropylene** test tubes for dilution of standards and samples
- Human Granulysin Controls (optional; R&D Systems, Catalog # QC327)

For cell lysates samples (optional):

- Lysis Buffer 17 (R&D Systems, Catalog # 895943)

PRECAUTIONS

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.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS 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.

Cell Lysates - Cells must be lysed prior to assay as directed in the Sample Values section.

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.*
Grossly icteric samples are not suitable for use in this assay.

SAMPLE PREPARATION

Use polypropylene tubes.

Due to matrix effects and endogenous levels, serum, plasma, supernates and lysate samples require a minimum 4-fold dilution. An example 4-fold dilution is 50 µL sample + 150 µL of Calibrator Diluent RD5-74.

For cell lysate samples, quantitation of sample protein concentration using a total protein assay is recommended. The suggested range for total cell lysate protein added is 0.5-5 µg/well.

Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

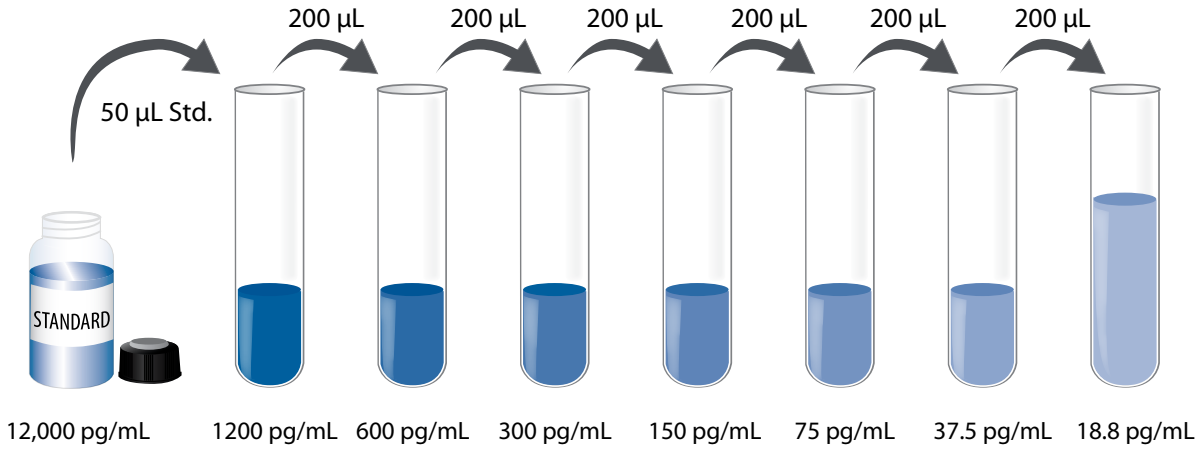
Human Granulysin Capture Antibody Concentrate - Refer to the vial label for reconstitution volume. Reconstitute the Human Granulysin Capture Ab Concentrate with Assay Diluent RD1-111. This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to rest for a minimum of 5 minutes with gentle agitation before diluting. The 20X capture antibody stock can be stored for up to 4 weeks at 2-8 °C once reconstituted.

Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-111. For a full plate, combine 300 µL of the reconstituted Human Granulysin Capture Ab stock and 300 µL of Human Granulysin Detection Ab Concentrate with 5.4 mL of Assay Diluent RD1-111 to create 6 mL of Human Granulysin Antibody Cocktail.

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 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Human Granulysin Standard - **Refer to vial label for reconstitution volume.** Reconstitute the Human Granulysin Standard with Calibrator Diluent RD5-74. This reconstitution produces a stock solution of 12,000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions. Use the standard stock within 60 minutes.

Use polypropylene tubes. Pipette 450 µL of Calibrator Diluent RD5-74 into the 1200 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1200 pg/mL standard serves as the high standard. Calibrator Diluent RD5-74 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, samples, and working standards 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 50 µL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12″ orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 100 µL of TMB ELISA Substrate solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. Add 100 µL of ELISA 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.
8. 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 require dilution. See Sample Preparation Section.

CALCULATION OF RESULTS

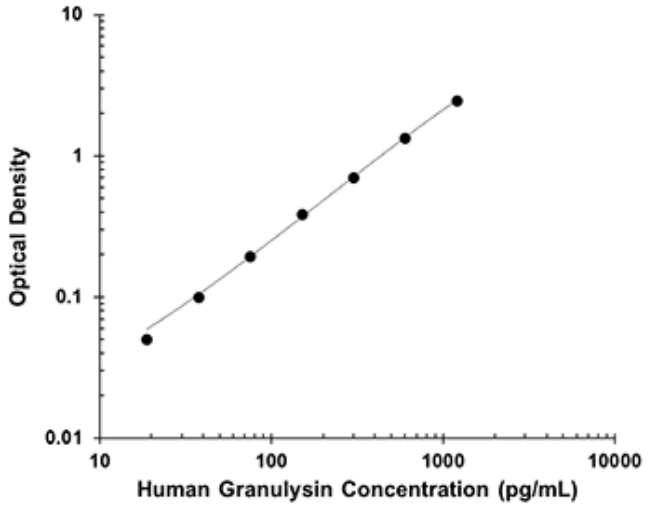
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 Granulysin 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

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.021 0.023	0.022	—
18.8	0.070 0.073	0.072	0.050
37.5	0.118 0.126	0.122	0.100
75.0	0.214 0.219	0.217	0.195
150	0.397 0.419	0.408	0.386
300	0.725 0.733	0.729	0.707
600	1.359 1.374	1.367	1.345
1200	2.408 2.586	2.497	2.475

PRECISION

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

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least six technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (pg/mL)	110	636	87.1	571
Standard deviation	8.26	18.3	8.0	37.8
CV (%)	7.5	2.9	9.2	6.6

RECOVERY

The recovery of Granulysin is spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range %
Cell culture media (n=2)	110	97-131
Serum (n=2)	108	92-122
EDTA plasma (n=2)	96	84-106
Heparin plasma (n=2)	112	99-121
Lysis buffer (n=1)	106	96-114

SENSITIVITY

Fifteen assays were evaluated and the minimum detectable dose (MDD) of Granulysin ranged from 0.473-2.92 pg/mL. The mean MDD was 1.43 pg/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.

LINEARITY

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

		Cell culture supernates (n=2)	Serum (n=2)	EDTA plasma (n=2)	Heparin plasma (n=2)	Cell lysates (n=2)
1:2	Average % of Expected	92	101	95	99	94
	Range (%)	91-93	101-102	92-98	96-102	90-97
1:4	Average % of Expected	92	97	97	98	96
	Range (%)	90-94	94-100	93-100	97-100	92-100
1:8	Average % of Expected	86	96	94	95	91
	Range (%)	86-87	93-98	94-95	93-98	86-95
1:16	Average % of Expected	88	93	92	93	95
	Range (%)	88-88	90-96	92-92	91-96	93-97

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human Granulysin produced at R&D Systems™.

SAMPLE VALUES

Serum/plasma - Samples from apparently healthy volunteers were evaluated for the presence of Granulysin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	1291	855-2229	377
EDTA plasma (n=10)	1306	894-2167	364
Heparin plasma (n=10)	1214	852-1873	303

Cell culture supernates/lysates – Human peripheral blood mononuclear cells (PBMCs; single donor) (1 x 10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were stimulated with 10 µg/mL PHA for 48 hours. Aliquots of the cell culture supernates were collected, centrifuged to remove cells and debris, and assayed for levels of human Granulysin.

Sample	(pg/mL)
PBMC 1	4677
PBMC 2	3336

For the cell culture lysates, centrifuged cells were washed with PBS before solubilizing the cells in Lysis Buffer 17 (Catalog # 895943) with protease inhibitors using 3-5X the cell pellet volume and assayed for levels of human Granulysin.

Sample	(pg/mL)
PBMC 3	1084
PBMC 4	623

SPECIFICITY

This assay recognizes natural and recombinant human Granulysin.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a low level recombinant human Granulysin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CD-8

Fas

Fas Ligand

Granzyme A

Granzyme B

Granzyme H

CD94

Perforin-1

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.