QUANTITATIVE DETERMINATION OF HUMAN GRANULYSIN

NEW PRODUCT

Human Granulysin ELISA

- Sensitivity (0.03 ng/ml)
- Very good analytical characteristics
- Validated for human serum, plasma (EDTA, citrate, heparin)
- Preliminary population data

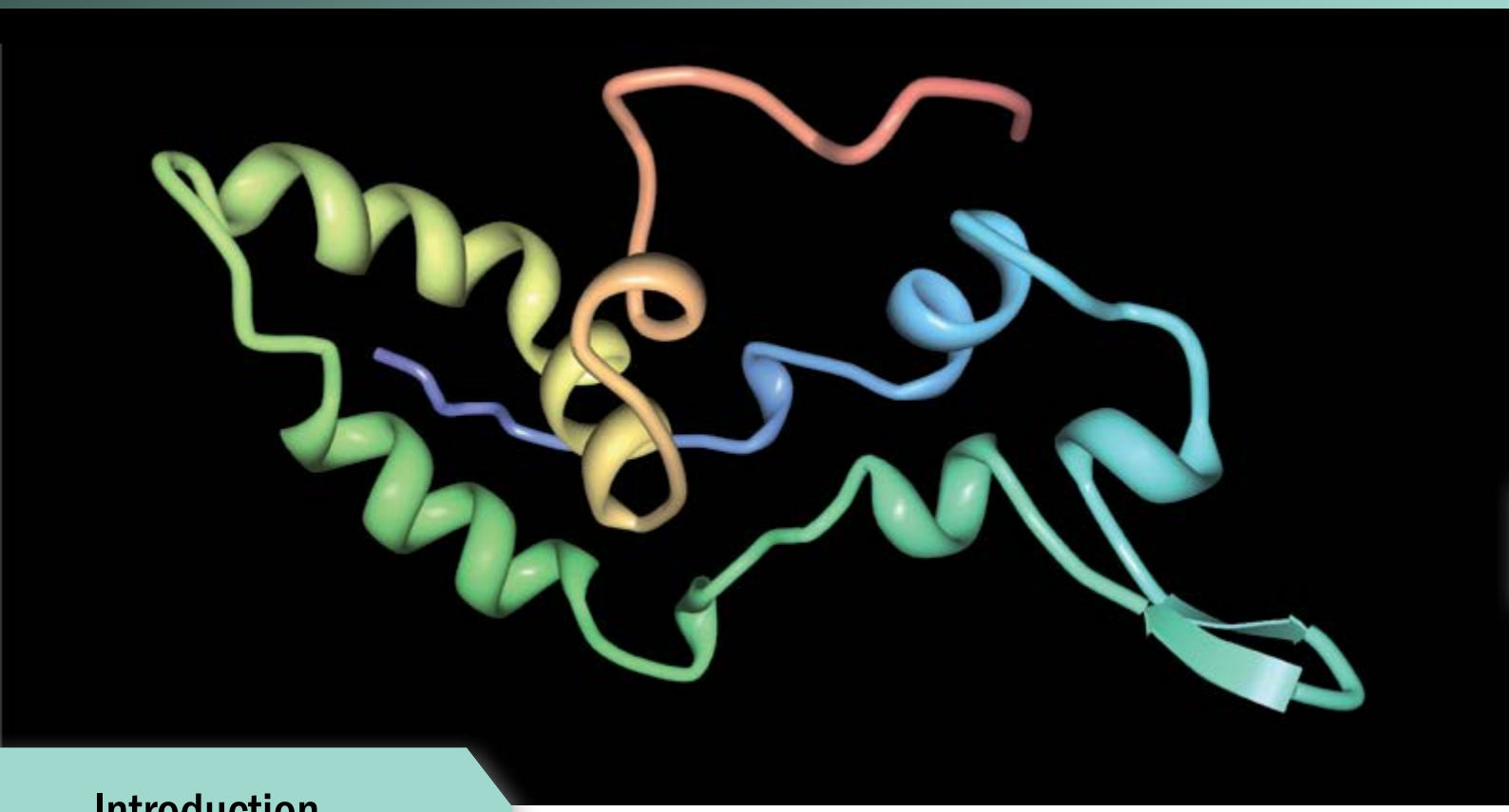
IMMUNE RESPONSE, INFECTION AND INFLAMMATION

ONCOLOGY

TRANSPLANTATION

BioVendor
Research
and Diagnostic Products

HUMAN GRANULYSIN ELISA



Introduction

Granulysin was first identified by subtractive hybridization in a search for genes expressed by human T lymphocytes 'late' (3–5 days) after activation [1]. Granulysin is a product of protein-coding gene with the same name – Granulysin, composed of 5 exons [2]. This protein is a member of the saposin-like protein family (SAPLIP) which contain a saposin B-type domain. A single mRNA is translated into 15 kDa granulysin, some of which is processed at both the amino and carboxy termini to a 9 kDa protein [3]. The two molecules differ in their roles in immune responses and cell localization. The 9 kDa form is sequestered in cytolytic granules and rapidly released after degranulation, while the 15 kDa form is constitutively secreted [4]. Both isoform induce expression of proinflammatory cytokines, act as chemoattractants or alarmins and activate immature dendritic cells (iDC).

Studies with recombinant 9 kDa granulysin have demonstrated its cytolytic and proinflammatory properties [3]. 9 kDa granulysin is contained in the cytotoxic granules of cytolytic T-cells (CTLs) and natural killers (NKs) and it is directionally released following target cell recognition. This isoform is proinflammatory and has a broad cytotoxic spectrum against gram-negative and gram-positive bacteria, fungi, yeast, parasites and tumors [3, 5, 6, 10, 11, 12, 13]. Granulysin contributes to apoptosis of cancer cells via Cytochrome C. Granulysin directly kills Mycobacterium tuberculosis and is involved in host defence against leprosy. Recombinant 9 kDa granulysin is dependent on perforin for killing intracellular pathogens. Perforin and granzyme B are colocalized with 9 kDa granulysin. Granulysin is an important mediator of damage in a variety of skin diseases, including folliculitis, psoriasis, acne, lichen planus and viral vesicles.

Recent data also suggest that granulysin may be useful as a diagnostic and therapeutic agent in clinical cancer [3, 5, 6]. Granulysin expression has been widely correlated with positive prognosis in variety of cancers. Progression of cancer was associated with decreased granulysin expression in peripheral NK cells in comparison to controls and tumor-free patients. Granulysin is suggested to be a potential biomarker in transplantation; its level increases in severity of graft vs host disease (GVHD) [7].

In contrast, 15 kDa granulysin is located in distinct granules negative for perforin and granzyme B (3) and these are released by activated cytolytic cells. The larger isoform is not cytotoxic, but plays an important role in differentiation of monocytes to dendritic cells. Further, 15 kDa granulysin activates both murine and human iDC.

Granulysin binds to and increases permeability of a target cell's plasma membrane resulting in a flux of calcium and potassium [7]. Blocking this ion flux protects cells from lysis. The granulysin-mediated increase of intracellular calcium could contribute to mitochondrial damage and induction of apoptosis. Indeed, granulysin has been shown to damage

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the mitochondrial membrane in the presence of calcium, and cause the release of cytochrome C and production of reactive oxygen species, and in addition to inducing the permeability of lysosomal membranes. It also may induce damage of the endoplasmic reticulum in a caspase 7-dependent manner and contribute to the activation of caspase 3.

No specific receptor for granulysin has been identified to date, however, granulysin has been postulated to activate a G-coupled protein receptor and TLR4 (Toll-like receptor 4), at least in a mouse model [8]. Because mice do not express granulysin or an apparent homologue, transgenic mice for human granulysin must be created to establish in vivo activity [9]. In vivo, mice expressing granulysin show markedly improved anti-tumor responses, with an increased numbers of activated dendritic cells and cytokine-producing T cells [3].

Current knowledge suggests that the distinct functions of granulysin isoforms have major implications for diagnosis and potential new therapies for human disease.

BioVendor Human Granulysin ELISA (RD191327200R)

Intended use

The RD191327200R Human Granulysin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of native human granulysin (GNLY).

- The total assay time is less than 3,5 hours
- The kit measures total human granulysin in serum, plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard is purified recombinant protein
- Components of the kit are provided ready to use, concentrated or lyophilized

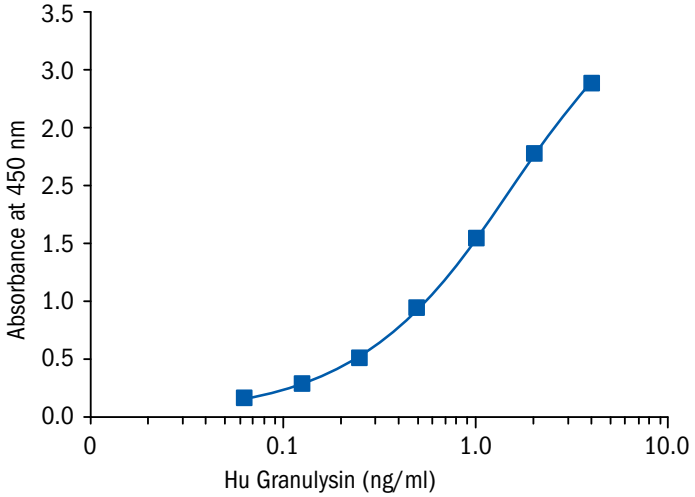
Clinical application

- Immune Response, Infection and Inflammation
- Oncology
- Transplantation

HUMAN GRANULYSIN ELISA CAT. NO.: RD191327200R	
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit
Samples	Serum, Plasma (EDTA, citrate, heparin)
Standards	0.063 to 4 ng/ml
Limit of detection	0.03 ng/ml

Test principle

In the BioVendor Human Granulysin ELISA, standards and samples are incubated in a microtitrate plate wells pre-coated with polyclonal anti-human granulysin antibody. After 60 minutes incubation and a washing, biotin-labelled polyclonal anti-human granulysin antibody is added and incubated with captured granulysin for 60 minutes. After another washing, the streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration granulysin. A standard curve is constructed by plotting absorbance values against concentrations of granulysin standards, and concentrations of unknown samples are determined using this standard curve.



Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
Serum 1	0.8	0.03	3.6
Serum 2	1.93	0.09	4.5

Inter-assay (Run-to-Run) (n=5)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
Serum 1	0.82	0.03	4.0
Serum 2	1.88	0.17	9.0

Spiking recovery

Serum samples were spiked with different amounts of human granulysin and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	0.52	-	-
	0.81	0.83	96.7
	1.10	1.15	96.1
	1.76	1.77	99.2
Serum 2	0.93	-	-
	1.2	1.24	96.6
	1.56	1.55	100.3
	2.19	2.18	100.5

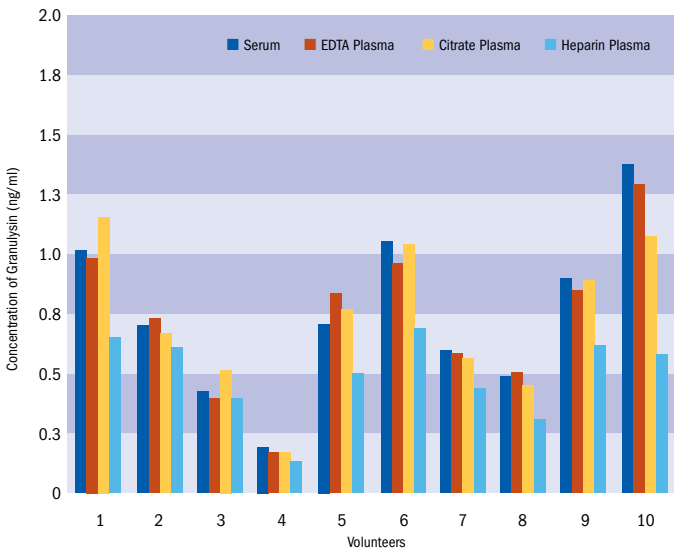
Linearity

Serum and plasma samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	-	2.63	-	-
	2x	1.28	1.31	97.3
	4x	0.65	0.66	98.4
	8x	0.33	0.33	100.5
Serum 2	-	2.99	-	-
	2x	1.47	1.49	98.2
	4x	0.76	0.75	101.2
	8x	0.39	0.37	103.2
Plasma EDTA	-	1.55	-	-
	2x	0.73	0.78	94.2
	4x	0.35	0.39	89.0
	8x	0.16	0.19	82.6
Plasma heparin	-	0.88	-	-
	2x	0.43	0.44	97.1
	4x	0.18	0.22	82.3
	8x	0.10	0.11	91.4
Plasma citrate	-	1.27	-	-
	2x	0.60	0.64	93.7
	4x	0.25	0.32	77.2
	8x	0.11	0.16	66.1

Effect of sample matrix

EDTA, citrate and heparin plasma samples were compared to respective serum samples from the same 10 individuals. Results are shown below:



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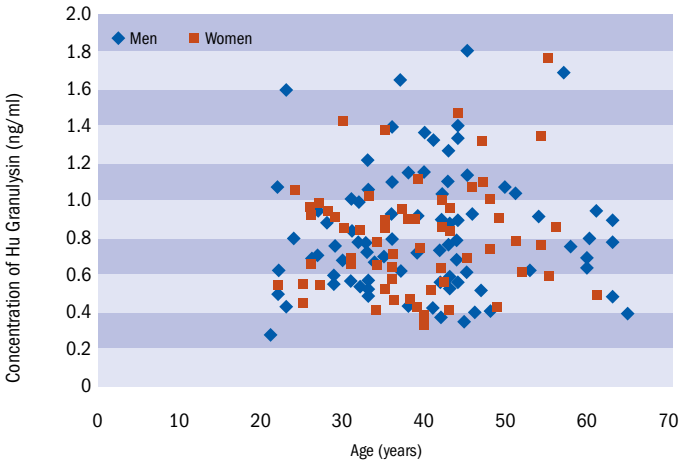
Preliminary Population Data

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 21–65 years old were assayed with the BioVendor Human Granulysin ELISA in our laboratory:

Age and Sex Dependent Distribution of Granulysin

Sex	Age (years)	n	Mean Granulysin (ng/ml)	Median Granulysin (ng/ml)	SD Granulysin (ng/ml)	Min. Granulysin (ng/ml)	Max. Granulysin (ng/ml)
Men	20 - 29	18	0.73	0.69	0.30	0.28	1.60
	30 - 39	26	0.83	0.77	0.29	0.42	1.64
	40 - 49	31	0.85	0.86	0.36	0.35	1.82
	50 - 65	14	0.83	0.78	0.30	0.39	1.69
Women	20 - 29	12	0.79	0.91	0.21	0.44	1.06
	30 - 39	26	0.77	0.72	0.26	0.40	1.44
	40 - 49	20	0.78	0.79	0.32	0.32	1.47
	50 - 61	8	0.90	0.77	0.41	0.48	1.76



Summary of protocol

- Reconstitute Master Standard and prepare set of Standards
- Dilute samples (serum/plasma 5x)
- Add 100 µl Standards and samples
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Biotin Labelled Antibody solution
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Streptavidin – HRP Conjugate
- Incubate at RT for 30 minutes/300 rpm
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl Stop Solution
- Read absorbance and calculate results

Related products

- RD172327100 Granulysin Human *E. coli*

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