

## PRODUCT DATASHEET

### Granulysin Human E. coli

**Cat. No.:** RD172327100

**Type:** Recombinant protein

**Size:** 0.1 mg

**Source:** E. coli

**Species:** Human

#### Description

Total 133 AA. MW: 15.33 kDa (calculated). UniProtKB acc.no. P22749 (Arg23-Leu145). N-Terminal His-tag (10 extra AA). Protein identity confirmed by LC-MS/MS.

#### Other names

Lymphokine LAG-2, T-cell activation protein 519, GNLY, LAG2, NKG5, TLA519

**Introduction to the molecule**

Granulysin was first identified by subtractive hybridization in a search for genes expressed by human T lymphocytes 'late' (3– 5 days) after activation. Granulysin is a product of protein-coding gene with the same name – Granulysin, composed of 5 exons. 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. 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. 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. 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. 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. 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). In contrast, 15 kDa granulysin is located in distinct granules negative for perforin and granzyme B 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. 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 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. Because mice do not express granulysin or an apparent homologue, transgenic mice for human granulysin must be created to establish in vivo activity. In vivo, mice expressing granulysin show markedly improved anti-tumor responses, with an increased numbers of activated dendritic cells and cytokine-producing T cells. Current knowledge suggests that the distinct functions of granulysin isoforms have major implications for diagnosis and potential new therapies for human disease.

**Research topic**

Animal studies, Immune Response, Infection and Inflammation, Oncology, Transplantation

**Amino Acid sequence**

MKHHHHHHAS RLSPEYYDLA RAHLRDEEKS CPCLAQEGPQ GDLTKTQEL GRDYRTCLTI VQKLKKMVDK PTQRSVSNAATRVCRGTGRSR WRDVCNRFMR RYQSRVTQGL VAGETAQQIC EDLRLCIPST GPL

**Purity**

Purity as determined by densitometric image analysis: >95%

**Endotoxin**

< 0.1 EU/μg

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**Formulation:**

Filtered (0.4 µm) and lyophilized in 0.5 mg/mL in 50 mM phosphate buffer, 300 mM NaCl, 5% (w/v) trehalose, pH 7.5.

**Reconstitution:**

Add deionized water to prepare a working stock solution of 0.5 mg/mL and let the lyophilized pellet dissolve completely. Product is not sterile! Please filter the product by an appropriate sterile filter before using it in the cell culture.

**Shipping**

At ambient temperature. Upon receipt, store the product at the temperature recommended below.

**Storage, Stability/Shelf Life**

Store the lyophilized protein at -80 °C. Lyophilized protein remains stable until the expiry date when stored at -80 °C. Aliquot reconstituted protein to avoid repeated freezing/thawing cycles and store at -80 °C for long term storage. Reconstituted protein can be stored at 4 °C for a week.

**Quality control**

BCA to determine quantity of the protein.

SDS PAGE to determine purity of the protein.

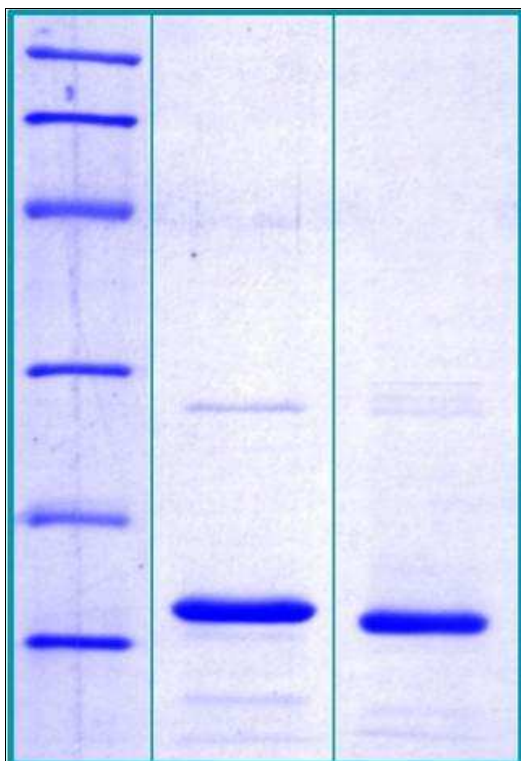
LAL to determine quantity of endotoxin.

**Applications**

ELISA, Western blotting

**Note**

This product is intended for research use only.



14% SDS-PAGE separation of Hu Granulysin:

1. M.W. marker – 97, 66, 45, 31, 21, 14 kDa
2. reduced and heated sample, 2.5μg/lane
3. non-reduced and non-heated sample, 2.5μg/lane