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ProductInformation

Granzyme A
murine, recombinant
expressed in *Pichia pastoris*

Product Number **G 6041**
Storage Temperature –20 °C

EC 3.4.21.78
Synonym: GzmA

Product Description
Granzyme A (GzmA) is the second most prominent granzyme in a family of 11 found in the cytotoxic granules, secreted by cytotoxic T lymphocytes (CTL) and natural killer (NT) cells. The granzymes enter the target cell with the assistance of perforin, a critical molecule of the cytotoxic granules. In the target cell the granzymes act on specific substrates involved with the cell death via apoptosis.[1,2] Granzyme A functions as a slow acting apoptotic enzyme providing a "back up" system when granzyme B is inhibited in the target cell by serpin like inhibitors. These inhibitors are introduced to the cells via viral infections.[3,4] Granzyme A has been shown to cleave proteins such as interleukin 1β,[5] type IV collagen,[6] thrombin receptor,[7] and pHAP;[8] however, the physiological substrates of Granzyme A are still unknown.

Granzyme A, a 232 amino acid protein, is synthesized as a preproenzyme, containing a leader sequence, which is cleaved by a signal peptidase, and a two amino acid prodomain, which is cleaved by the lysosomal cysteine protease DPPI. It is glycosylated with mannose-rich carbohydrates, which are important for its packaging into lytic granules. Granzyme A forms a disulfide-linked homodimer and its native MW is 60 kDa.[2]

Murine, recombinant Granzyme A is expressed in *Pichia pastoris* as a mature active protein. It appears on reducing SDS-PAGE as a doublet of approximately 30 and 32 kDa and on non-reducing SDS-PAGE as doublet of 60-70 kDa. The doublet originates for different glycosylations. Murine, recombinant Granzyme A activity is similar to the native Granzyme A activity.[3] Granzyme A is a trypsin-like serine protease (tryptase), which cleaves synthetic substrates with Lys or Arg at the P1 position.

The product is supplied as a solution in 50 mM Hepes, pH 7.5, containing 150 mM NaCl.

Purity: minimum 90% (SDS-PAGE). A doublet is observed due to different glycosylations at approximately 32 and 30 kDa.

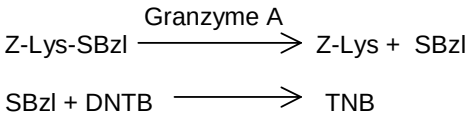
Specific Activity: minimum 1,000 units per mg protein

Unit Definition: The amount of enzyme that hydrolyzes 1 nmole of Z-Lys-SBzl per minute at pH 7.5 at 30 °C.

Precautions and Disclaimer
This product is for laboratory use only, not for drug, household, or other use. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
This product ships on wet ice and storage at –20 °C is recommended. Avoid freeze-thaw cycles

Procedure
Granzyme A hydrolyzes the synthetic substrate Z-Lys-SBzl with the release of the thiobenzyl group. The free thiobenzyl group reacts with DTNB [5,5'-dithiobis(2-nitrobenzoic acid) or Ellman's reagent] and produces a chromophore which absorbs at 412 nm.



Stock solutions and reagents

Note: Prepare with ultrapure water

- 1x Phosphate buffered saline, pH 7.5.
- 11 mM DTNB Solution: Dissolve 4.36 mg of DTNB (Product No. D 8130, MW = 396.4) in 1 ml of ethanol. Store at –20 °C.
- Prepare 1 ml of 10% TRITON™ X-100.
- DMSO
- Z-Lys-SBzl hydrochloride

Working solutions

Note: Prepare with ultrapure water

- Assay Buffer: 1x PBS, pH 7.5, with 0.11 mM DTNB and 0.01% TRITON X-100 (See Table 1)

Table 1.

Preparation of 10 ml of Assay Buffer

Reagent	Concentration of stock solution	Final concentration	Amount per 10 ml
PBS	1x	1x	9.9 ml
TRITON X-100	10%	0.01%	10 µl
DTNB	11 mM	0.11 mM	0.1 ml

- Substrate Solution: 10 mM Z-Lys-SBzl hydrochloride (MW = 423.0) in DMSO. Dissolve 4.23 mg of Z-Lys-SBzl hydrochloride in 1 ml of DMSO. Store at –20 °C.
- Enzyme Solution: Dilute Granzyme A with 1x PBS to approximately 0.025 mg/ml.

Equipment

- Spectrophotometer with temperature control
- 1 ml quartz cuvette

Assay Procedure

It is advisable to perform the assay in duplicate.

1. Set the spectrophotometer to measure absorbance at 412 nm.
2. Set temperature to 30 °C.
3. Preincubate the Assay Buffer at 30 °C.
4. Add the appropriate amount of Assay Buffer to cuvette (see Table 2).

Table 2.

Reaction scheme

Test	Assay Buffer	Granzyme A Solution	10 mM Z-Lys-SBzl
1 (control)	970 µl		30 µl
2	950 µl	20µl	30 µl
3	930 µl	40µl	30 µl

5. For the control, add 30 µl of Substrate Solution, mix by inversion, and read using a kinetic program (20 second intervals for 2 to 3 minutes). Note: The substrate hydrolyzes spontaneously without the presence of the enzyme during the incubation period, therefore, the control is necessary.
6. For testing enzyme activity, add the appropriate amount of diluted Enzyme Solution (see Table 2), add 30 µl of Substrate Solution, mix by inversion, and read using a kinetic program (20 second intervals for 2 to 3 minutes).
7. Calculate Granzyme A activity.

Calculation

- Unit definition: 1 unit of Granzyme A will hydrolyze 1.0 nmole of Z-Lys-SBzl per minute at 30 °C at pH 7.5.
- ε of TNB at 412 nm = 0.012 cm^{-1} μM^{-1}
- R - Reaction volume = 1 ml
- V - Volume of Enzyme Solution per test in ml
- D – Dilution factor for preparing Enzyme Solution
- C – Concentration in mg of protein per ml of the original enzyme solution.
- Calculate (ΔA_{sample} - $\Delta A_{control}$) per minute.
- Calculate Granzyme A activity in units per mg of protein

$$\text{Units/mg-P} = \frac{(\Delta A_{sample} - \Delta A_{control}) \times D \times R}{0.012 \times V \times C}$$

References

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