3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Granzyme B from mouse
recombinant, expressed in *Pichia pastoris*

Catalog Number **G9278**
Storage Temperature –70 °C

Synonym: GzmB

Product Description
Cytotoxic granules secreted by natural killer (NK) cells and cytotoxic T Lymphocytes cells are part of the mechanism used for protecting the organism from virus infected and tumor cells. Granzyme B, a serine protease, is the most prominent granzyme in a family of 11 found in the cytotoxic granules. 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 cell death via apoptosis.

Granzyme B is a neutral serine protease that cleaves after aspartic acid residues, inducing cell death by various pathways.[1-3] It can cleave and activate most of the caspases *in vitro* and *in vivo* resulting in a massive amplification of the caspase dependent apoptotic pathway. In addition, granzyme B directly cleaves downstream caspase substrates, PARP DNA-PK2 and DFF45/ICAD,[4] leading to cell death. This pathway bypasses inhibition of apoptosis by viral caspase inhibitors found in virus infected cells. It was shown granzyme B is capable of inducing cytochrome C release from the mitochondria in a caspase independent way.[5]

Granzyme B is a 247 amino acid polypeptide 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.[6]

This recombinant granzyme B is expressed in *Pichia pastoris* as the mature form and appears on SDS-PAGE as a triplet (~34, 32, and 30 kDa) due to three different glycosylations.[7]

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

Recombinant Granzyme B expressed in *Pichia pastoris* is a soluble secreted form of the active enzyme with the same specificities as native Granzyme B. This recombinant, active, mature Granzyme B can cleave caspase 3 both *in vitro*[7] and *in vivo*[4] to its signature p20/p10 forms.

Recombinant Granzyme B expressed in *Pichia pastoris* has the advantage over native Granzyme B, as the latter can only be purified in limited amounts, and over *E. coli* and *vaccinia* virus expressed enzymes that failed to generate soluble active enzyme.

Purity: ≥90% (SDS-PAGE). A triplet due to three different glycosylations (~34, 32, and 30 kDa)

Specific activity: ≥10,000 units/min/mg

Unit definition: One unit will hydrolyze 1 nmol of Boc-Ala-Ala-Asp-SBzl per min at pH 7.5 at 30 °C.

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

Storage/Stability
Store the product at –70 °C. Avoid freeze and thaw cycles.

Procedure
Principle of assay
Granzyme B hydrolyzes the substrate Boc-Ala-Ala-Asp-sBz and releases the thiobenzyl group. The thiobenzyl group reacts with DTNB [Ellman's reagent, 5,5′-dithio-bis(2-nitrobenzoic acid)] and produces a chromophore, which absorbs light at 412 nm.

Stock solutions

Note: Use ultrapure water (≥18 MΩ×cm resistivity at 25 °C) for the preparation of reagents.

- 1 M Hepes, pH 7.5
- 0.5 M EDTA, pH 7.5
- 3 M NaCl
- 11 mM DTNB (Catalog Number D8130) – Dissolve 4.36 mg of DTNB in 1 ml of ethanol.
- 10 mM Boc-Ala-Ala-Asp-SBzl (Catalog Number B5305) in DMSO (100×) – Dissolve 5 mg in 1 ml of dry DMSO.

Working solutions

Note: Use ultrapure water (≥18 MΩ×cm resistivity at 25 °C) for the preparation of reagents.

- Assay buffer – 100 mM Hepes, pH 7.5, with 0.3 M NaCl, 1 mm EDTA, and 0.11 mM DTNB.
- Enzyme formulation buffer – 50 mM Hepes, pH 7.5, with 150 mM NaCl
- Granzyme B – 0.025–0.035 mg/ml. Dilute Granzyme B with formulation buffer to obtain the recommended concentration.

Equipment

- Spectrophotometer, heat regulated to 30 °C, set to 412 nm.
- 0.5 ml quartz cuvette
- Detection 412 nm, kinetic mode, 60 second interval without delay.

Reaction scheme

	Assay buffer	100× Substrate	Granzyme B
Blank	500 µl	10 µl	
Sample	500 µl	10 µl	10 µl

Assay

1. Set the spectrophotometer in a kinetic mode at 412 nm, 60 second interval without delay. The linear range, which gives the highest activity, is within the first minute.
2. Pre-warm the spectrophotometer and the assay buffer to 30 °C.
3. Zero the spectrophotometer on the assay buffer.
4. Blank – Add 500 µl of Assay buffer to the cuvette, add 10 µl of 10 mM substrate, mix immediately by inversion, put the cuvette in the spectrophotometer, and start recording absorbance at 412 nm.
5. Sample – Add 500 µl of assay buffer to the cuvette, add 10 µl of diluted enzyme, mix, add 10 µl of 10 mM substrate, mix immediately by inversion, put the cuvette in the spectrophotometer, start recording absorbance at 412 nm.
6. Calculate Granzyme B activity.

Note: Some substrate decomposition occurs during the incubation period, therefore:

1. The blank test is obligatory.
2. The assay can be performed only in a cuvette, as a one-at-a-time reaction, and not in a multiwell plate format.

Results

Calculate ΔA_{412}/60 second for Granzyme B sample (ΔS) and for the blank reaction (ΔB), by subtracting the zero point A_{412} from the 60 second A_{412}.

Calculate the amount of enzyme per test in mg (mg-P/test)

Calculate Granzyme B activity (Units/mg-P)

$$\text{Units/mg-P} = \frac{(\Delta S - \Delta B) \times 0.5}{0.012 \times \text{mg-P/test}}$$

where:
$\varepsilon_{\mu M}^{412}$ of TNB – 0.012
Reaction volume – 0.5 ml

Unit definition – One unit will hydrolyze 1 nmol of Boc-Ala-Ala-Asp-SBzl per min at pH 7.5 at 30 °C.

References

1. Shresta, S. et al., Curr. Opin. Immunol., **10**, 581-587 (1998)
2. Trapani, J.A. et al., Immunology Today, **20**, 351-356, 1999.
3. Trapani, J.A. et al., Curr. Opin. Immunol., **12**, 323-329 (2000)
4. Thomas, D.A. et al., Immunity, **12**, 621-632 (2000)
5. Heibein, J.A. et al., J. Immunol., **163**, 4663-4693 (1999)
6. Smyth, M.J. et al., J. Immunol., **154**, 6299-6305 (1995)
7. Pham, C.T.N., J. Biol. Chem., **273**, 1629-1633 (1998)

NDH,PHC,MAM 06/14-1