Recombinant Human Granzyme A

Catalog Number: 2905-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human Granzyme A protein Cys26-Val262, with a C-terminal 10-His tag Accession # P12544
N-terminal Sequence Analysis	Cys26
Structure / Form	Pro form
Predicted Molecular Mass	28 kDa

SPECIFICATIONS

SDS-PAGE	Multiple bands between 29-33 kDa, reducing conditions
Activity	Measured by its ability to cleave a colorimetric peptide substrate, N-carbobenzyloxy-Gly-Arg-ThioBenzyl ester (Z-GR-SBzl), in the presence of 5,5'Dithio-bis (2-nitrobenzoic acid) (DTNB). Edwards, K.M. *et al.* (1999) J. Biol. Chem. **274**:30468. The specific activity is >5,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in MES, NaCl and $CaCl_2$ with Trehalose. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 0.1 M Tris, pH 9.0
- Assay Buffer: 50 mM Tris, pH 8.0
- Recombinant Human Granzyme A (rhGranzyme A) (Catalog # 2905-SE)
- Lysyl-Endopeptidase
- Substrate: Z-Gly-Arg-thiobenzyl ester, 10 mM stock in DMSO
- 5,5'Dithio-bis(2-nitrobenzoic acid) (DTNB), 10 mM stock in DMSO
- Clear 96-well Plate
- Plate Reader with Absorbance Read Capability

Assay

1. Activate rhGranzyme A at 50 µg/mL with 0.1 µg/mL Lysyl-Endopeptidase in Activation Buffer.
2. Incubate at 37 °C for 1 hour.
3. Dilute activated rhGranzyme A to 0.2 ng/µL in Assay Buffer.
4. Dilute Substrate to 200 µM in Assay Buffer containing 200 µM of DTNB.
5. In a plate, load 50 µL of 0.2 ng/µL rhGranzyme A, and start the reaction by adding 50 µL of 200 µM Substrate mixture. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of Substrate mixture.
6. Read absorbance at a wavelength of 405 nm, in kinetic mode for 5 minutes.
7. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 13260 $M^{-1}cm^{-1}$

***Using the path correction 0.320 cm

Note: the output of many spectrophotometers is in mOD.

Final Assay Conditions

Per Well:

- rhGranzyme A: 0.01 µg
- DTNB: 100 µM
- Substrate: 100 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 25 mM HEPES, 150 mM NaCl and 10 mM $CaCl_2$, pH 7.5.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

Granzyme A is a member of the granzyme family of the serine proteases found specifically in the cytotoxic granules of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. Granzyme A is the most abundant protease in CTL and NK cells. It induces caspase-independent cell death when introduced into target cells by perforin (1). Human granzyme A is synthesized as a precursor (262 residues) with a signal peptide (residues 1-26), a propeptide (residues 27-28) and a mature chain (residues 29-262) (2). The purified recombinant human Granzyme A consists of residues 26 to 262. After being activated by lysyl endopeptidase, it cleaves a thioester substrate as described in Activity Assay Protocol.

References:

1. Lieberman, J. and Z. Fan (2003) Curr. Opin. Immunol. **15**:553.
2. Gershenfeld, H.K. *et al.* (1988) Proc. Natl. Acad. Sci. USA **85**:1184.

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