Source
Mouse myeloma cell line, NS0-derived human Granzyme B protein
Gly19-Tyr247, with a C-terminal 10-His tag
Accession # P10144

N-terminal Sequence Analysis
Gly19

Structure / Form
Pro form

Predicted Molecular Mass
27 kDa

SPECIFICATIONS

SDS-PAGE
30-40 kDa, reducing conditions

Activity
Measured by its ability to cleave a peptide substrate, t-Butyloxycaronyl-Ala-Ala-Asp-ThioBenzyl ester (Boc-AAD-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 >1,000 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
Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials
- Activation Buffer: 50 mM MES, 50 mM NaCl, pH 5.5
- Assay Buffer: 50 mM Tris, pH 7.5
- Recombinant Human Granzyme B (rhGranzyme B) (Catalog # 2906-SE)
- Recombinant Mouse Active Cathepsin C/DPPI (rmCathepsin C) (Catalog # 2336-CY)
- Substrate: t-Butyloxycaronyl-Ala-Ala-Asp-ThioBenzyl ester (SM Biochemicals LLC, Catalog # SMSB05), 10 mM stock in DMSO
- 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) (Sigma, Catalog # D-8130), 10 mM stock in DMSO
- (optional) E-64 (Tocris, Catalog # 5208), 50 mM stock in DMSO
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay
1. Activate rhGranzyme B at 100 µg/mL with 10 µg/mL active rmCathepsin C in Activation Buffer.
2. Incubate at 37 °C for 4 hours. (Optional: use 10 μM E-64 in activation buffer to stop activating enzyme, this is not required under these conditions).
3. Dilute activated rhGranzyme B to 0.5 ng/µL in Assay Buffer.
4. Dilute Substrate to 200 µM in Assay Buffer containing 200 µM DTNB.
5. In a plate load 50 µL of 0.5 ng/µL rhGranzyme B. Include a Substrate Blank containing 50 µL of Assay Buffer.
6. Start the reaction by adding 50 µL of Substrate mixture to wells.
7. Read in kinetic mode for 5 minutes at an absorbance of 405 nm.
8. Calculate specific activity:

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\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}} \times (\text{OD/min}) \times \text{well volume (L)} \times 1 \times 10^{12} \text{pmol/M}}{\text{ext. coeff}^\ast \times (M^{-1}cm^{-1}) \times \text{path corr.}^\ast\ast \times (cm) \times \text{amount of enzyme (µg)}}
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*Adjusted for Substrate Blank
**Using the extinction coefficient 13260 M⁻¹cm⁻¹
***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions Per Well:
- rhGranzyme B: 0.025 µg
- DTNB: 100 µM
- Substrate: 100 µM

PREPARATION AND STORAGE

Shipping
The product is shipped with dry ice or equivalent. 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.
Granzyme B 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 (1, 2). Granzyme B plays an essential role in granule-mediated apoptosis and may have additional roles in rheumatoid arthritis and in bacterial and viral infections (3). It activates various caspases and cleaves proteins such as aggrecan (3). Human Granzyme B is synthesized as a precursor (247 residues) with a signal peptide (residues 1-18), a pro peptide (residues 19-20), and a mature chain (residues 21-247) (4-6). The rhGranzyme B consisting of residues 19-247 was expressed and purified. After being activated by active cathepsin C, rhGranzyme B cleaves a thioester substrate described previously (3).

References: