

**DESCRIPTION**

<b>Source</b>	Mouse myeloma cell line, NS0-derived human Granzyme B protein Gly19-Tyr247, with a C-terminal 10-His tag Accession # P10144
<b>N-terminal Sequence Analysis</b>	Gly19
<b>Structure / Form</b>	Pro form
<b>Predicted Molecular Mass</b>	27 kDa

**SPECIFICATIONS**

<b>SDS-PAGE</b>	30-40 kDa, reducing conditions
<b>Activity</b>	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. <i>et al.</i> (1999) J. Biol. Chem. <b>274</b> :30468. The specific activity is >1,000 pmol/min/μg, as measured under the described conditions.
<b>Endotoxin Level</b>	<1.0 EU per 1 μg of the protein by the LAL method.
<b>Purity</b>	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
<b>Formulation</b>	Supplied as a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

**Activity Assay Protocol**

<b>Materials</b>	<ul style="list-style-type: none"> <li>• Activation Buffer: 50 mM MES, 50 mM NaCl, pH 5.5</li> <li>• Assay Buffer: 50 mM Tris, pH 7.5</li> <li>• Recombinant Human Granzyme B (rhGranzyme B) (Catalog # 2906-SE)</li> <li>• Recombinant Mouse Active Cathepsin C/DPPI (rmCathepsin C) (Catalog # 2336-CY)</li> <li>• Substrate: t-Butyloxycaronyl-Ala-Ala-Asp-ThioBenzyl ester (SM Biochemicals LLC, Catalog # SMSB05), 10 mM stock in DMSO</li> <li>• 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) (Sigma, Catalog # D-8130), 10 mM stock in DMSO</li> <li>• (optional) E-64 (Tocris, Catalog # 5208), 50 mM stock in DMSO</li> <li>• 96-well Clear Plate (Costar, Catalog # 92592)</li> <li>• Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent</li> </ul>
------------------	---

<b>Assay</b>	<ol style="list-style-type: none"> <li>1. Activate rhGranzyme B at 100 μg/mL with 10 μg/mL active rmCathepsin C in Activation Buffer.</li> <li>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).</li> <li>3. Dilute activated rhGranzyme B to 0.5 ng/μL in Assay Buffer.</li> <li>4. Dilute Substrate to 200 μM in Assay Buffer containing 200 μM DTNB.</li> <li>5. In a plate load 50 μL of 0.5 ng/μL rhGranzyme B. Include a Substrate Blank containing 50 μL of Assay Buffer.</li> <li>6. Start the reaction by adding 50 μL of Substrate mixture to wells.</li> <li>7. Read in kinetic mode for 5 minutes at an absorbance of 405 nm.</li> <li>8. Calculate specific activity:</li> </ol>
--------------	---

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

\*Adjusted for Substrate Blank

\*\*Using the extinction coefficient 13260 M<sup>-1</sup>cm<sup>-1</sup>

\*\*\*Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

<b>Final Assay Conditions</b>	<p>Per Well:</p> <ul style="list-style-type: none"> <li>• rhGranzyme B: 0.025 μg</li> <li>• DTNB: 100 μM</li> <li>• Substrate: 100 μM</li> </ul>
-------------------------------	--

**PREPARATION AND STORAGE**

<b>Shipping</b>	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<p><b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b></p> <ul style="list-style-type: none"> <li>• 6 months from date of receipt, -70 °C as supplied.</li> <li>• 3 months, -70 °C under sterile conditions after opening.</li> </ul>

## BACKGROUND

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:

1. Kam, C.-M. *et al.* (2000) *Biochim. Biophys. Acta* **1477**:307.
2. Smyth, M.J. *et al.* (1996) *J. Leukoc. Biol.* **60**:555.
3. Froelich, C.J. (2004) in *Handbook of Proteolytic Enzymes*, Barrett, A.J. *et al.*, eds., pp. 1549.
4. Schmid, J. and C. Weissman (1987) *J. Immunol.* **139**:250.
5. Caputo, A. *et al.* (1988) *J. Biol. Chem.* **263**:6363.
6. Trapani, J.A. *et al.* (1988) *Proc. Natl. Acad. Sci. USA* **85**:6924.