R&D SYSTEMS
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# Recombinant Human Transglutaminase 7/TGM7

Catalog Number: 5426-TG

## DESCRIPTION

| | |
|---|---|
| **Source** | *Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived<br>Asp2-Pro710, with an N-terminal Met and 6-His tag<br>Accession # Q96PF1 |
| **N-terminal Sequence Analysis** | No result obtained |
| **Predicted Molecular Mass** | 81 kDa |

## SPECIFICATIONS

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| **SDS-PAGE** | 75-78 kDa |
| **Activity** | Measured by its ability to form CBZ-Gln-Gly-Hydroxamate from CBZ-Gln-Gly and Hydroxylamine.<br>The specific activity is >350 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** | >90%, by SDS-PAGE under reducing conditions and visualized by silver stain. |
| **Formulation** | Supplied as a 0.2 µm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details. |

## Activity Assay Protocol

**Materials**

- Recombinant Human Transglutaminase 7/TGM7 (rhTGM7) (Catalog # 5426-TG)
- Substrate: Z-Gln-Gly (Sigma, Catalog # C6154), 500 mM (dissolve in deionized water, then adjust to pH 9.0 with NaOH)
- 0.1 M MES, pH 6.0
- Dithiothreitol (DTT), 1 M stock in DMSO
- 1.0 M $CaCl_2$
- 1.0 M Hydroxylamine Hydrochloride (Sigma, Catalog # 159417) (Dissolve in deionized water, then adjust to pH 6.0 with NaOH)
- Stop Solution: 0.37 M $FeCl_3$ (Sigma, Catalog # 236489), 0.67 M HCl, 0.2 M Trichloroacetic Acid
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

**Assay**

1. Prepare substrate mixture right before running assay. Mix the following components per reaction:
   a. 15 µL 500 mM Z-Gln-Gly
   b. 75 µL 400 mM MES, pH 6.0
   c. 7.5 µL 200 mM DTT
   d. 7.5 µL 200 mM $CaCl_2$
   e. 15 µL 1 M Hydroxylamine Hydrochloride
   Note: Multiply the volume for each component by the number of reaction vials + 1 to make enough substrate mixture for the assay. (For example: If there are 4 reaction vials, including blanks, multiply all volumes by 5)
2. Dilute rhTGM7 to 0.1 mg/mL in deionized water.
3. Mix 30 µL of the diluted rhTGM7 with 120 µL substrate mixture (step #1). Include a Substrate Blank containing 30 µL deionized water and 120 µL substrate mixture.
4. Incubate at 37 °C for 2 hours.
5. After incubation, stop the reaction with 600 µL of the Stop Solution. Mix well.
6. Centrifuge at top speed for 2 minutes in a microcentrifuge.
7. Transfer 200 µL (in duplicate) of the supernatant into a plate.
8. Read at 525 nm (absorbance) in endpoint mode.
9. Calculate specific activity:

$$\text{Specific Activity (pmoles/min/µg)} = \frac{\text{Adjusted Abs* (OD) x Conversion Factor** (pmole/OD)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard L-glutamic acid g-monohydroxamate (Sigma, Catalog # G2253).

**Final Assay Conditions**

Per Well:

- rhTGM7: 0.8 µg
- Substrate: 10 mM

## PREPARATION AND STORAGE

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| **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.**<br>• 6 months from date of receipt, -70 °C as supplied.<br>• 3 months, -70 °C under sterile conditions after opening. |



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## BACKGROUND

Transglutaminase 7 (TG7), encoded by the TGM7 gene, is also known as protein-glutamine-g-glutamyltransferase Z (Tgase Z) (1). It belongs to the family of Transglutaminases that catalyze the posttranslational modification of proteins via calcium dependent cross-linking reactions (2-4). TG7 is ubiquitously expressed in humans (1). Members of the TGM family have been implicated in a variety of human diseases including neurodegenerative diseases, celiac disease, lamellar ichthyosis, bleeding disorders, cataract formation, atherosclerosis, and others (5).

**References:**

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4. Griffin, M. *et al.* (2002) Biochem. J. **368**:377.
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