R&D SYSTEMS
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Recombinant Human Transglutaminase 2/TGM2

Catalog Number: 4376-TG

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Ala2-Ala687 (Asn533Thr and Leu655Val), with an N-terminal Met and 6-His tag Accession # P21980
N-terminal Sequence Analysis	No results obtained
Predicted Molecular Mass	78 kDa

SPECIFICATIONS

SDS-PAGE	82 kDa, reducing conditions
Activity	Measured by its ability to cleave a synthetic peptide Benzyloxycarbonyl-Gln-Gly and NH_2OH. The specific activity is >1000 pmol/min/ug, as measured under the described condition.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Diluent: deionized water
- Recombinant Human Transglutaminase 2/TGM2 (rhTGM2) (Catalog # 4376-TG)
- Substrate: Z-Gln-Gly (Sigma, Catalog # C6154), dissolve 500 mM in deionized water, then neutralize it to pH 9.0 with NaOH
- MES, pH 6.0, 1 M stock
- DTT (Sigma, Catalog # D-0632), 1 M stock in deionized water
- $CaCl_2$,1 M stock in deionized water
- 1 M Hydroxylamine Hydrochloride (Sigma, Catalog # 159417), dissolve in deionized H_2O, then neutralize it 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 fresh before running assay. Mix the following components (Dilute all components to the correct concentration in Assay Diluent.):
   a. 10 µL of 500 mM Substrate
   b. 50 µL of 400 mM MES, pH 6.0
   c. 5 µL of 200 mM DTT
   d. 5 µL of 200 mM $CaCl_2$
   e. 10 µL of 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 assays. For example: If there are 2 reaction vials (including blank), multiply all volumes by 3.)
2. Dilute rhTGM2 to 0.1 mg/mL in Assay Diluent.
3. Mix 20 µL of the diluted rhTGM2 with 80 µL Substrate Mixture. For the Substrate Blank mix 20 µL of Assay Diluent with 80 µL Substrate Mixture.
4. Incubate at 37 °C for 2 hours.
5. After incubation, stop the reaction with 400 µL of the Stop Solution. Mix well.
6. Centrifuge at top speed for 2 minutes in a microcentrifuge.
7. Load 200 µL of the supernatant into a plate.
8. Read at 525 nm (absorbance) in endpoint mode.
9. Calculate specific activity:

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

*Adjusted for Substrate Blank
**Derived using calibration standard L-glutamic acid γ-monohydroxamate (Sigma, Catalog # G2253).

Final Assay Conditions

Per Well:

- rhTGM2: 0.8 µg
- Substrate: 10 mM

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.

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BACKGROUND

Transglutaminase 2 (TG2), encoded by the TGM2 gene, is also known as tissue transglutaminase (tTG), transgluytaminase C (TGC), and protein-glutamine-γ-glutamyltransferase. It belongs to the family of transglutaminases that catalyze the posttranslational modification of proteins via calcium dependent cross-linking reactions (1-3). In addition to its function in protein cross-linking, TGM2 is also capable of hydrolyzing both GTP and ATP (4) and has intrinsic kinase activity (5). TGM2 has been implicated in a variety of human diseases including celiac disease, inclusion body myositis, atherosclerosis, and neurodegenerative diseases (6, 7).

References:

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