Recombinant Human Glutaminyl-peptide Cyclotransferase/QPCT

Catalog Number: 6368-ZN

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human Glutaminyl-peptide Cyclotransferase/QPCT protein Ala33-Leu361, with an N-terminal 6-His tag Accession # Q16769
N-terminal Sequence Analysis	His
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

SDS-PAGE	36-40 kDa, reducing conditions
Activity	Measured by its ability to convert Glutaminyl-AMC to pyroglutamyl-AMC. The specific activity is >550 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM HEPES, pH 7.0
- rhPGPEP-1 Buffer: 0.1 M Tris, 5 mM DTT, pH 9.0
- Recombinant Human Glutaminyl-peptide Cyclotransferase/QPCT (rhQPCT) (Catalog # 6368-ZN)
- Substrate: Q-AMC (Bachem, Catalog # I-1175)
- Recombinant Human PGPEP-1 (rhPGPEP-1) (Catalog # 6278-CY)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhQPCT to 0.2 µg/mL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Combine 150 µL of 0.2 ug/mL rhQPCT and 150 µL of 200 µM Substrate. Include a Substrate Blank containing 150 µL Assay Buffer and 150 µL of 200 µM Substrate.
4. Incubate reaction at room temperature for 20 minutes.
5. Stop reaction by boiling at 100 °C for 5 minutes and cool on ice for 3 minutes.
6. Dilute rhPGPEP-1 to 1 µg/mL in rhPGPEP-1 Buffer.
7. Add 300 µL of 1 µg/mL rhPGPEP-1 to each vial.
8. Incubate reaction at room temperature for 10 minutes.
9. Load 100 µL in quadruplicate from each vial into a 96 well plate.
10. Read plate at excitation and emission wavelengths of 380 nm and 460 nm, respectively, in endpoint mode.
11. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- rhQPCT: 0.005 µg
- Substrate: 50 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. 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 opening.

BACKGROUND

Glutaminyl-peptide Cyclotransferase, also known as Glutaminyl Cyclase, catalyzes the conversion of N-terminal L-glutaminyl residues of peptides to pyroglutamyl groups (1). The enzyme is present in the pituitary and adrenal glands, where it is important for the generation of the N-terminal pyroglutamyl groups of peptide hormones such as neurotensin and thyrotropin-releasing hormone. Glutaminyl Cyclase also catalyzes the conversion of N-terminal L-glutamyl residues to pyroglutamyl residues (2). This activity may contribute to the formation of several amyloid-related plaque forming peptides, contributing to Alzheimer's disease pathology. Glutaminyl Cyclase is also considered to be a diagnostic marker of thyroid tumors (3).

References:

1. Busby, W.H. Jr. *et al.* (1987) J. Biol. Chem. **262**:8532.
2. Schilling, S. *et al.* (2004) FEBS Lett. **563**:191.
3. Griffith, O.L. *et al.* (2006) J. Clin. Oncol. **24**:5043.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449