Recombinant Human γ-Glutamylcyclotransferase/CRF21

Catalog Number: 5086-CT

DESCRIPTION

Source	*E. coli*-derived Met1-Leu188 Accession # O75223
N-terminal Sequence Analysis	Ala2
Predicted Molecular Mass	21 kDa

SPECIFICATIONS

SDS-PAGE	20-22 kDa, reducing conditions
Activity	Measured by its ability to release L-alanine from γ-glutamyl-L-alanine, with the detection of alanine by alanine dehydrogenase. The specific activity is >40,000 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 under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 9.0
- Recombinant Human γ-Glutamylcyclotransferase/CRF21 (rhCRF21) (Catalog # 5086-CT)
- γ-L-Glutamyl-L-alanine (Sigma, Catalog # 483834), 100 mM stock in deionized water
- β-Nicotinamide adenine dinucleotide (β-NAD) (Sigma, Catalog # N6522), 100 mM stock in deionized water
- L-Alanine Dehydrogenase (Sigma, Catalog # A7653)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhCRF21 to 0.05 ng/µL in Assay Buffer.
2. Prepare a Substrate Mixture containing 10 mM γ-L-Glutamyl-L-alanine, 2 mM β-NAD, and 0.008 units/µL L-Alanine Dehydrogenase in Assay Buffer.
3. Load into a plate 50 µL of 0.05 ng/µL rhCRF21, and start the reaction by adding 50 µL of Substrate Mixture. For Substrate Blanks, load 50 µL of Assay Buffer and 50 µL of Substrate Mixture.
4. Read plate at a wavelength of 340 nm (bottom read) in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank
**Using the extinction coefficient 6220 $M^{-1}cm^{-1}$
***Using the path correction 0.32 cm
Note: the output of many spectrophotometers is in mOD.

Final Assay Conditions

- hCRF-21: 0.0025 µg
- γ-L-Glutamyl-L-alanine: 5 mM
- β-NAD: 1 mM
- L-Alanine Dehydrogenase: 0.4 units

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.

BACKGROUND

Gamma-glutamylcyclotransferase (GGCT) is an essential enzyme in the γ-glutamyl cycle that catalyzes the formation of 5-oxoproline from gamma-glutamyl dipeptides. The enzyme plays an important role in glutathione homeostasis. GGCT is also known as cytochrome c-releasing factor 21(CRF-21). It induces the release of cytochrome c from mitochondria, leading to apoptosis (1). It is a 188 amino acid protein that consists of six β-strands, five α-helices and four short 3_{10} helices (2). Up-regulation of the expression of GGCT has been found in various tumor tissues including lung, esophagus, stomach, bile duct, uterine cervix, colon and breast. (3, 4). Therefore, GGCT is a potential biomarker for numerous types of cancers.

References:

1. Masuda Y. *et al.* (2006) Biochem. Biophys. Res. Commun. **346**:454.
2. Oakley A.J. *et al.* (2008) J. Biol. Chem. **283**:22031.
3. Gromov P. *et al.* (2010) J. Proteome Res. **9**:3941.
4. Amano T. *et al.* (2012) J. Histochem. Cytochem. **60**:76.

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