

DESCRIPTION

Source *E. coli*-derived
Met1-Asp260, with a C-terminal 6-His tag
Accession # NP_001035517

N-terminal Sequence Analysis Met1

Predicted Molecular Mass 30 kDa

SPECIFICATIONS

SDS-PAGE 29-30 kDa, reducing conditions

Activity Measured by its ability to hydrolyze S-lactoylglutathione.
The specific activity is >25,000 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 >85%, 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 250 mM NaCl, pH 7.5
- Recombinant Human Glyoxalase II (rhGlyoxalase II) (Catalog # 5944-GO)
- Substrate: S-Lactoylglutathione (Sigma, Catalog # L7140), 100 mM stock in deionized water
- 5, 5'-dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma, Catalog # D8130), 10 mM stock in DMSO
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhGlyoxalase II to 0.4 ng/μL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer with 400 μM DTNB to form Substrate Mixture.
3. Load 50 μL of 0.4 ng/μL rhGlyoxalase II into a plate and start the reaction by loading 50 μL of Substrate Mixture. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of Substrate Mixture.
4. Read at 405 nm (absorbance) in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Using the extinction coefficient 13260 M⁻¹cm⁻¹

***Using the path correction 0.320 cm

Note: the output of many spectrophotometers is in mOD.

Final Assay Conditions

Per Well:

- rhGlyoxalase II: 0.020 μg
- S-Lactoylglutathione: 1 mM
- DTNB: 200 μM

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

The glyoxalase system consists of two enzymes, Glyoxalase I and Glyoxalase II. The purpose of the glyoxalase system is to detoxify α-ketoaldehydes, particularly methylglyoxal, a by-product of glycolysis (1). Glyoxalase II catalyzes the hydrolysis of S-lactoylglutathione, the product of the glyoxalase I reaction, to glutathione and lactate (2). Glyoxalase II exists as two isoforms, one cytosolic and one mitochondrial (3). The enzyme is believed to be important for the response of cells to stress (4).

References:

1. Thornalley, P.J. (1996) Gen. Pharmacol. **27**:565.
2. Ridderström, M. *et al.* (1996) J. Biol. Chem. **271**:319.
3. Cordell, P.A. *et al.* (2004) J. Biol. Chem. **279**:28653.
4. Xu, Y. and X. Chen (2006) J. Biol. Chem. **281**:26702.

PRODUCT SPECIFIC NOTICES

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