Recombinant Human Glutathione S-Transferase pi 1/GSTP1

Catalog Number: 6455-GT

DESCRIPTION

Source	*E. coli*-derived Met1-Glu210 Accession # AAC51280
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	23 kDa

SPECIFICATIONS

SDS-PAGE	24 kDa, reducing conditions
Activity	Measured by the conjugation of reduced glutathione to 1-bromo-2,4-dinitrobenzene. The specific activity is >25,000 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 100 mM NaH_2PO_4, pH 7.0
- Recombinant Human Glutathione S−Transferase pi 1/GSTP1 (rhGSTP1) (Catalog # 6455-GT)
- Substrate: 1-bromo-2,4-dinitrobenzene (BDNB) (Sigma, Catalog # 262226), 75 mM stock in ethanol
- L-Glutathione, reduced (GSH) (Amresco, Catalog # 399), 250 mM stock in deionized water
- UV Plate (Costar, Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhGSTP1 to 0.2 ng/µL in Assay Buffer.
2. Dilute GSH to 4 mM in Assay Buffer.
3. Combine equal volumes of 0.2 ng/µL rhGSTP1 and 4 mM GSH for 0.1 ng/µL rhGSTP1 with 2 mM GSH.
4. Dilute Substrate to 2 mM in Assay Buffer.
5. Load into a UV plate 50 µL of the rhGSTP1/GSH mixture. Include a substrate blank containing 25 µL of Assay Buffer with 25 µL of the 4 mM GSH prepared in step 2.
6. Start the reaction by adding 50 µL of 2 mM Substrate to well.
7. Read in kinetic mode for 5 minutes at an absorbance of 340 nm.
8. Calculate specific activity:

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

*Adjusted for Substrate Blank
**Using the extinction coefficient 9600 $M^{-1}cm^{-1}$
***Using the path correction 0.320 cm
Note: the output of many spectrophotometers is in mOD.

Final Assay Conditions

Per Well:

- rhGSTP1: 0.005 µg
- GSH: 1 mM
- Substrate: 1 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

Glutathione S-Transferases (GSTs) are members of the phase II detoxification enzyme family that conjugate glutathione to various electrophilic compounds, including metabolites generated by oxidative processes in the body, environmental toxins or carcinogens, and anti-cancer drugs. GSTP1 is a cytosolic protein that belongs to pi class of the GST superfamily. It is crystallized as a homodimer (1), but also exists in solution as an equilibrium mixture of monomer and dimer, depending on the protein concentration (2). Four genetic variants of GSTP1 with different enzymatic activities have been identified, which indicates the particular allelic form expressed in tissues could contribute to variation in catalytic efficiency and biological functions (3, 4). Human GSTP1 is present at elevated levels in many tumor cells, and has unique properties as a cancer marker (5). Genetic polymorphisms and expression patterns of GSTP1 have been associated with a variety of effects on human cancer, anti-cancer drug resistance, and asthma (6). In addition to its role as a drug-metabolizing enzyme, GSTP1 has ligand binding properties and regulates kinase signaling pathways through protein-protein interactions (7).

References:

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PRODUCT SPECIFIC NOTICES

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