

DESCRIPTION

Source *E. coli*-derived human Glutathione Reductase protein
Met44-Arg522, with an N-terminal Met and 6-His tag
Accession # P00390

N-terminal Sequence Analysis Met

Predicted Molecular Mass 53 kDa

SPECIFICATIONS

SDS-PAGE 53 kDa, reducing conditions

Activity Measured by its ability to reduce oxidized glutathione to glutathione in an NADPH-dependent manner.
The specific activity is >15,000 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, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 0.1 M Tris, pH 7.5
 - Recombinant Human Glutathione Reductase (rhGSR) (Catalog # 8866-GR)
 - NADPH (Sigma, Catalog # N7505), 10 mM stock in deionized water
 - Oxidized Glutathione (Amresco, Catalog # 0524), 50 mM stock in deionized water
 - 96-well Clear Plate (Catalog # DY990)
 - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

- Assay**
- Create Substrate Mixture containing 0.2 mM NADPH and 1 mM Oxidized Glutathione in Assay Buffer.
 - Dilute rhGSR to 0.4 μg/mL in Assay Buffer.
 - Load 50 μL of the 0.4 μg/mL rhGSR to the plate, and start the reaction by adding 50 μL of Substrate Mixture. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of Substrate Mixture.
 - Read plate in kinetic mode for 5 minutes at an absorbance of 340 nm.
 - The specific activity can be calculated using the following equation:

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

*Adjusted for Substrate blank

**Using the extinction coefficient 6220 M⁻¹cm⁻¹

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD.

- Final Assay Conditions**
- Per Well:
- rhGSR: 0.02 μg
 - NADPH: 0.1 mM
 - Oxidized Glutathione: 0.5 mM

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

Glutathione Reductase (GR) is a homodimeric flavoprotein disulfide oxidoreductase that plays a critical role in the prevention of oxidative damage within the cell (1, 2). Glutathione Reductase helps to maintain appropriate levels of intracellular reduced glutathione (GSH) which is essential for maintaining the normal structure of red blood cells and for keeping hemoglobin in the ferrous state (3). Glutathione Reductase together with its co-factor, NADPH, catalyzes the reduction of oxidized glutathione (glutathione disulfide, GSSG) to glutathione. GSH is also a reactant for Glutathione Peroxidase, which converts hydrogen peroxide (H₂O₂) into water (4). Glutathione Reductase is expressed as both mitochondrial and cytosolic isoforms, as determined by the presence or absence of an N-terminal transit peptide. Human Glutathione Reductase shares 86% and 76% amino acid sequence identity with mouse and rat Glutathione Reductase, respectively (5, 6). Alternative splicing of human Glutathione Reductase generates additional isoforms with deletions in the central pyridine nucleotide-disulfide oxidoreductase domain (7).

References:

1. Deponte, M. (2013) *Biochim. Biophys. Acta* **1830**:3217.
2. Fujii, J. *et al.* (2011) *J. Clin. Biochem. Nutr.* **49**:70.
3. Tsantes, A.E. *et al.* (2006) *Antioxid. Redox Signal.* **8**:1205.
4. Lubos, E. *et al.* (2011) *Antioxid. Redox Signal.* **15**:1957.
5. Tutic, M. *et al.* (1990) *Eur. J. Biochem.* **188**:523.
6. Krauth-Siegel, R.L. *et al.* (1982) *Eur. J. Biochem.* **121**:259.
7. Satoh, N. *et al.* (2010) *Biochem. Genet.* **48**:816.