

Recombinant Human Glyoxalase II

Catalog Number: 5944-GO

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: Specific Activity (pmol/min/μg) = Adjusted V _{max} * (OD/min) x well volume (L) x 10 ¹² pmol/mol ext. coeff** (M-¹cm⁻¹) x path corr.*** (cm) x amount of enzyme (μg) *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: • InGlyoxalase 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 • 6 months from date of receipt, -70 °C as supplied.	DESCRIPTION	
Accession # NP_001035517 Metaminal Sequence Analysis Predicted Molecular M	Source	E. coli-derived
Machaysis Precification Moses Predicted Molecular Mass SDS-PAGE 29-30 kDa, reducing conditions Activity Measured by its ability to hydrolyze S-lactolyglutathione. The specific activity is 25-50.000 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 Glycvalsae II (ricGlycvalsae) II) (Catalog # 5944-GO) • Substrate: S-Lactolyglutathione (Sigma, Catalog # L7140), 100 mM stock in DMSO • So-well Clear Plate (Costar, Catalog # 2592) • Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent Assay 1. Dilute Glycvalsae II to 0.4 ng/Li. in Assay Buffer. 2. Dilute Substrate to 2 mM in Assay Suffer with 400 μM DTNB to form Substrate Mixture. 2. Dilute Substrate to 2 mM in Assay Suffer with 400 μM DTNB to form Substrate Mixture. 3. Load 50 μ, 6 of Assay Buffer and 50 μ, of Substrate Mixture. 4. Read at 405 m (absorbance) in kinetic mode for 5 minutes. 5. Calculate specific activity: Specific Activity (pmol/min/μg) = Adjusted V _{max} * (OD/min) x well volume (L) x 10 ¹² pmol/mol ext. coeff** (M*1cm*1) x path corr.*** (cm) x amount of enzyme (μg) **Adjusted for Substrate Blank** **Using the extinction coefficient 13260 M*1cm*1 ***Using		· ·
Predicted Molecular Mass		-
SPECIFICATIONS		Met1
Activity Measured by its ability to hydrolyze S-lactoylglutathione. The specific activity is z-25,000 pmol/min/µg, as measured under the described conditions. Endotoxin Level 4.10 EU per 1 µg of the protein by the LAL method.		30 kDa
SDS-PAGE 29-30 kDa, reducing conditions	SPECIFICATIONS	
The specific activity is >25,000 pmol/min/μg, as measured under the described conditions. Endotoxin Level		29-30 kDa, reducing conditions
Purity	Activity	
Supplied as a 0.2 μm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details. Activity Assay Protocol Materials	Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Materials • Assay Buffer: 50 mM Tris, 250 mM NaCl, pH 7.5 • Recombinant Human Glyoxalase II (rhGlyoxalase II) (Catalog # 5944-GO) • Substrate: S-Lactoy/glutathione (Sigma, Catalog # L7140), 100 mM stock in deionized water • 5, 5'-dithiols(2-nitrobenzoic acid) (DTNB) (Sigma, Catalog # D1830), 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 fh/Glyoxalase 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. 4. Read at 405 mm (absorbance) in kinetic mode for 5 minutes. 5. Calculate specific activity: Specific Activity (pmol/min/μg) = Adjusted V _{max} * (OD/min) x well volume (L) x 10 ¹² pmol/mol ext. coeff** (M*cm*¹) x path corr.*** (cm) x amount of enzyme (μg) *Adjusted for Substrate Blank **Using the extinction coefficient 13260 M*lcm*¹ **Using the extinction coefficient 13260 M*lcm*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.	Purity	>85%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
## Assay Buffer: 50 mM Tris, 250 mM NaCl, pH 7.5 ## Recombinant Human Glyoxalase II (rinGlyoxalase II) (Catalog # 5944-GO) ## Substrate: S-Lactoylglutathione (Sigma, Catalog # 1740), 100 mM stock in deionized water ## 5, 5-dithiobis(2-nitrobenzole acid) (DTNB) (Sigma, Catalog # 26792) ## Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent ## Assay ## 1. Dilute rinGlyoxalase 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 rinGlyoxalase 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 mm (absorbance) in kinetic mode for 5 minutes. ## 5. Calculate specific activity: ## Specific Activity (pmol/min/μg) =	Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.
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Recombinant Human Glyoxalase II (rhGlyoxalase II) (Catalog # 5944-GO) Substrate: S-Lactoylglutathione (Sigma, Catalog # L7140), 100 mM stock in deionized water 5, 5'-dithlobis[2-hirtobenzoic acid) (DTMB) (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: Specific Activity (pmol/min/μg) = Adjusted V _{max} * (OD/min) x well volume (L) x 10 ¹² pmol/mol ext. coeff** (M¹-cm¹) x path corr.*** (cm) x amount of enzyme (μg) *Adjusted for Substrate Blank ***Using the extinction coefficient 13260 M⁻-cm¹ ***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. Use a manual defrost freezer and avoid repeated freeze-thaw cycles. • 6 months from date of receipt, -70 °C as supplied.		
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: Specific Activity (pmol/min/μg) = Adjusted V _{max} * (OD/min) x well volume (L) x 10 ¹² pmol/mol ext. coeff** (M-¹cm-¹) x path corr.*** (cm) x amount of enzyme (μg) *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 PTREPARATION 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.		 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)
Using the extinction coefficient 13260 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: • 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.	Assay	 Dilute Substrate to 2 mM in Assay Buffer with 400 μM DTNB to form Substrate Mixture. 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. Read at 405 nm (absorbance) in kinetic mode for 5 minutes. Calculate specific activity: Adjusted V _{max} * (OD/min) x well volume (L) x 10 ¹² pmol/mol
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To individual forms of the conditions after opening.		Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

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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