Recombinant *F. graminearum* Galactose Oxidase His-tag

Catalog Number: 11206-GX

DESCRIPTION

Source	*E. coli*-derived *f. graminearum* Galactose Oxidase protein Ala42-Gln680, with a C-terminal 6-His tag Accession # I1S2N3.1
N-terminal Sequence Analysis	Ala42
Predicted Molecular Mass	70 kDa

SPECIFICATIONS

SDS-PAGE	60-68 kDa, under reducing conditions.
Activity	Measured by its ability to oxidize galactose. The specific activity is >75,000 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
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 Sodium Phosphate, pH 7.0
- Recombinant *F. graminearum* Galactose Oxidase His-tag (rF. GalOx) (Catalog # 11206-GX)
- D-(+)-Galactose, 750 mM stock in deionized water
- Horseradish Perosidase (HRP), 250 U/mL stock in 0.1 M Sodium Phosphate, pH 8.0
- 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 50 mM stock in deionized water
- Hydrogen Peroxide, 30% in water
- Copper (II) Sulfate, 1 M stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader

Assay

1. Dilute rF. GalOx to 0.3 ng/µL in Assay Buffer.
2. Activate rF. GalOx by adding 1 mM Copper (II) Sulfate and incubate on ice for 30 minutes.
3. Dilute 30% Hydrogen Peroxide 1/4080 using deionized water. Then, dilute the Hydrogen Peroxide 1/10 using Assay Buffer. This is the first point of the standard curve.
4. Complete the standard curve by performing six one-half serial dilutions using Assay Buffer. The standard curve has a range of 0.625 to 40 nmol per well.
5. Prepare a Substrate Mixture containing 30 U/mL HRP, 8 mM ABTS, and 16 mM D-(+)-Galactose in Assay Buffer.
6. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
7. Load 50 µL of activated rF. GalOx into empty wells of the same plate as the curve. Include a Control containing 50 µL of Assay Buffer.
8. Start the reactions by adding 50 µL of substrate mixture to all wells, including standard curve wells and sample wells.
9. Seal and incubate plate at room temperature for 5 minutes.
10. Immediately read plate at 420 nm (absorbance) in endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{[\text{Adjusted Sample } OD_{420} \times \text{Conversion Factor}]^* \text{ (nmol)} \times (1000 \text{ pmol/nmol})}{\text{Incubation time (min)} \times \text{amount of enzyme (µg)}}$$

*Derived from the Hydrogen Peroxide standard curve using linear fitting and adjusted for Control

Final Assay Conditions

Per Well:

- rF. GalOx: 0.015 µg
- HRP: 15 U/mL
- ABTS: 4 mM
- D-(+)-Galactose: 8 mM

Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Recombinant *F. graminearum* Galactose Oxidase His-tag

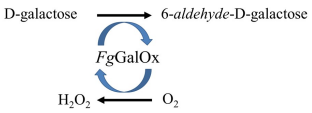
Catalog Number: 11206-GX

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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

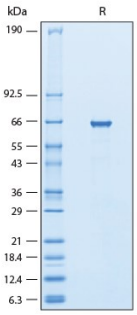
DATA

Enzyme Activity



Enzyme Reaction Scheme Recombinant F. graminearum Galactose Oxidase His-tag Protein (Catalog # 11206-GX) oxidizes free and terminal D-Galactose to 6-Aldehyde-D-Galactose with the concomitant production of H_2O_2 that allows convenient detection through horseradish peroxidase.

SDS-PAGE



Recombinant F. graminearum Galactose Oxidase His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant F. graminearum Galactose Oxidase His-tag Protein (Catalog # 11206-GX) was resolved with SDS-PAGE under reducing (R) conditions and visualized by Coomassie® Blue staining, showing a band at 65 kDa.

BACKGROUND

Fusarium graminearum galactose oxidase (GalOx) is an extracellular copper-containing oxidoreductase. Its catalytic site contains a free radical ligand formed by covalently cross-linked cysteine and tyrosine side chains coordinating to a copper center (1, 2, 3). GalOx catalyzes the two-electron oxidation of the C6-hydroxyl group of D-galactose as well as a range of primary alcohols to the corresponding aldehydes with concomitant reduction of oxygen to hydrogen peroxide. GalOx is strictly regioselective on primary alcohols and displays remarkable stereospecificity in its reaction with sugars, showing activity only with galactose but not glucose. Because of this specificity, GalOx is used to determine lactose levels in biological fluids, human milk and dairy products and examine histochemistry of mucus-secreting cells (4, 5). Furthermore, GalOx has been used to modify cell surface carbohydrates for cell labeling and histochemical staining and induce interferon in human lymphocyte culture (2, 6). GalOx is viewed as a competitive and cost-effective catalyst compared to chemical conversion for manufacturing fine chemicals in pharmaceutical and food industry. For example, GalOx is used for conversion of D-galactose to food-grade cross-linking agents (7).

References:

1. Baron, AJ. *et al.* (1994) J. Biol. Chem. **269**:25095.
2. Whittaker, J.W. (2002) Adv. Protein Chem. **60**:1.
3. Parikka K, *et al.* (2015) J. Mol. Catal., B Enzym. **120**:47.
4. Roberts, G.P. and Gupta, S.K. (1965) Nature **207**:425.
5. Kanyong, P. *et al.* (2013) Anal. Biochem. **435**:114.
6. Dianzani, F. *et al.* Infect. Immun. (1979) **26**:879.
7. Schoevaart, R. and Kieboom, T. (2001) Carbohydr. Res. **334**:1.

Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449