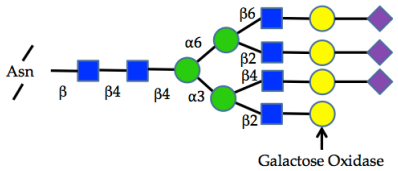
Product Specification Sheet

Product Name: Galactose Oxidase (EC 1.1.3.9)
Catalog Number: FSB0008
Lot Number: 140323

Description

Galactose Oxidase is a recombinantly expressed copper activated enzyme derived from *Dactylium Dendroides* that catalyzes the oxidation of primary alcohols to aldehydes and subsequently reduces dioxygen to hydrogen peroxide (1-3).



Product Information

Quantity:	50 Units
Specific Activity:	>1750 U/mg
Purity:	>95% by SDS-PAGE
Molecular Weight:	68.9 kDa
Ext. coefficient:	1.79 (1 mg/mL at 280 nm in H_2O)
Theoretical PI:	8.34
Storage:	**Buffer:** (50 mM Ammonium Acetate – pH 7.2, 10 µM $CuSO_4$) **Temperature:** 2-8°C. Avoid multiple freeze/thaw cycles. **Stability:** 3-6 months
Unit Definition:	One unit will produce a ΔA425 of 1.0 per min at pH 6.0 at 25 °C, in a peroxidase and o-Tolidine system.

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Activity Assay

Purpose: To quantify the amount of Units/mL in a galactose oxidase reaction using o-Tolidine (4).

Reagents:

Reaction Buffer	100 mM Potassium Phosphate pH 6.0
o-Tolidine	0.5% (w/v) o-Tolidine Solution (described below)
D-Galactose	10% (w/v) D-Galactose (in water)
Peroxidase	Horseradish Peroxidase (HRP) (5.0 Units/mL in Reaction Buffer)
GaO	Galactose Oxidase (0.5 Units/mL in Reaction Buffer)

Reagent Protocols:

o-Tolidine

Caution: o-Tolidine may be a carcinogen. Use appropriate precautions.

1. Dissolve 2 mg o-Tolidine in 0.4 mL methanol to create a 0.5% (w/v) solution.
2. Add the 0.5% (w/v) o-Tolidine solution to 47.6 mL of Reaction Buffer to create the working o-Tolidine solution.

Master Mix solution

This will create enough solution for 15 cuvette reactions or 300 reactions in a 96-well plate. Protect from light. The resulting o-Tolidine solution will oxidize relative quickly so it should be used in a few hours.

Reagent	Stock mL	Cuvette Assay mL	96-Well Assay µL
o-Tolidine	25		
10% D-Gal	22		
HRP	1.5		
Master	48.5	3.3	165
GaO	1.5	0.1	5
Total	50	3.4	170

Note: This assay is only linear near 0.05 Units/mL of Galactose Oxidase, therefore, it is vital that the assay be performed as close to this range as possible.

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Testing Protocol:

(1) Pre-warm the Master Mix, Galactose Oxidase solution and spectrophotometer to 20 C.

(a) *Cuvette Test (1 cm pathlength)*
Place 3.3 mL of the Master Mix in a cuvette. Add 0.1 mL of Galactose Oxidase solution, and mix.

(b) *Cuvette Test Blank (1 cm pathlength)*
Place 3.3 mL of the Master Mix in a cuvette. Add 0.1 mL of deionized water, and mix.

(2) Record the change in A425 nm for 300 sec (at intervals of 10 seconds or quicker).

(3) If the rate of change of the color is too rapid, then dilute the Galactose Oxidase solution appropriately.

Calibration Protocols:

Standard method to determine the units/mL of Galactose Oxidase (o-Tolidine)

$$\text{Units/mL Galactose Oxidase} = \frac{([\mathbf{\Delta A425/60sec}])(\mathbf{Vol\ Rxn})(\mathbf{DF})}{(\mathbf{Vol\ Enz})(\mathbf{SF})}$$

[ΔA425/60sec] = linear rate of change in A425 in 60 seconds of enzyme minus blank
Vol Rxn = volume of the reaction
Vol Enz = volume of enzyme
DF = dilution factor, which is experiment dependent.
SF = standardization factor for this reaction with o-Tolidine = 1

Specific Activity:

$$\text{Units/mg Galactose Oxidase} = \frac{\boldsymbol{Units/mL\ enzyme}}{\boldsymbol{concentration\ of\ enzyme}\ (\frac{\boldsymbol{mg}}{\boldsymbol{mL}})}$$

References

1. Avigard, G. *et al.* (1962) *J. Biol. Chem.* **237**, 2736-2743.
2. Schlegel, C.M. *et al.* (1968) *Carbohydr. Res.* **7**, 193-199.
3. Kosman, D. J. (1994) in *Copper Proteins and Copper Enzymes* Vol. 2, 1-26.
4. Yamamoto, K., *et al.* (1985) Agric. Biol. Chem. **49**, 2463 -2464.

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