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Not for use in diagnostic procedures.



β-Galactose Dehydrogenase **from *Pseudomonas fluorescens***

 **Version: 08**

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D-Galactose: NAD 1-oxidoreductase

Cat. No. 10 104 973 001 1 mg
 1 ml

Cat. No. 10 104 981 001 5 mg
 1 ml

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	β -Galactose Dehydrogenase	Suspension in 3.2 M ammonium sulfate solution, 1 mM EDTA, pH approximately 6.	10 104 973 001	1 vial, 1 ml
			10 104 981 001	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	β -Galactose Dehydrogenase	Store at +2 to +8°C.  A decrease in activity of approximately 10% occurs within 6 months.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Additional information

- The reaction of galactose dehydrogenase with glucose (a side reaction) proceeds at $\leq 0.1\%$ of the rate with galactose.
- Galactose dehydrogenase oxidizes both D-galactose and L-arabinose. To assay galactose specifically in a mixture of arabinose and galactose, use galactose oxidase or galactokinase. Assay of a galactose-arabinose mixture (a) with galactose oxidase (galactose only), then (b) with galactose dehydrogenase (both sugars), allows the determination of the amount of arabinose alone (result b minus result a).
- The gene for β -galactose dehydrogenase S was obtained from *Pseudomonas fluorescens* strain BMTU 102 and placed under the control of an *E. coli* β -galactosidase promoter in the plasmid pUR 51 for overproduction in *E. coli*.
- At pH 8.6, galactono- γ -lactone (product of galactose dehydrogenase) hydrolyzes spontaneously to galactonate, making the galactose dehydrogenase reaction at this pH essentially irreversible.
- To remove fluorescent, such as B vitamins, and quenching, such as phenol red substances which interfere with the fluorescent determination of low levels of lactose, pass the sample through a column of activated magnesium silicate (Florisil) before analysis.

2.2. Parameters

Activator

Unknown

Contaminants

<0.01% ADH and β -galactosidase each

<0.1% NADH oxidase

<0.5% LDH

EC-Number

EC 1.1.1.48

Inhibition

Unknown

Isoelectric Point

4.28

Molecular Weight

β -Galactose Dehydrogenase (64,000) is a dimer with subunits of equal size (subunit MW = 32,000 Da).

pH Optimum

The enzyme has a broad pH optimum in the alkaline pH range, which is dependent on the buffer used.

Buffer	Optimum pH
Bis(hydroxyethyl)amine	9.1 – 9.5
Tris-HCl	8.0 – 9.0
Pyrophosphate	8.6
Phosphate buffer	8.0 to 9.0
<i>i The enzyme retains 90% of optimal activity at pH 7.5.</i>	

Specific Activity

Approximately 5 U/mg protein at +25°C with D-galactose as the substrate, standardized with BSA.

Specificity

Substrate specificity and K_m

Galactose dehydrogenase will oxidize the following substrates:

Substrate	Relative rate
D-galactose (K_m : 0.7 mM at +30°C, pH 9.1)	1.00
L-arabinose	0.47
D-fucose	1.05
2-deoxy-D-galactose	0.27
2-amino-2-deoxy-D-galactose [galactosamine]	0.02, pH 8.6

Substrates not oxidized

L-galactose, D-arabinose, D-glucose, L-fucose, D-ribose, D-xylose, D-glucuronic acid, D-galacturonic acid, or D-galactose-6-phosphate.

The enzyme will use either NAD (K_m = 0.24 mM; relative rate = 1.0) or NADP (K_m = 2.3 mM; relative rate = 0.32).

Unit Definition

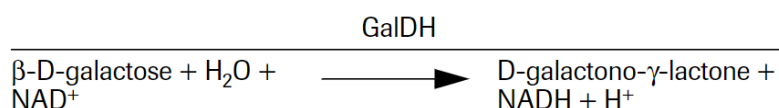
One unit β -Galactose Dehydrogenase will oxidize 1 μ mol of β -D-galactose in one minute at +25°C and pH 8.6. The assay produces 1 μ mol of NADH per μ mol of galactose oxidized.

3. Additional Information on this Product

3.1. Test Principle

Reaction mechanism

The assay described below produces 1 μ mol of NADH per μ mol of galactose oxidized. The oxidation of D-galactose to galactono- γ -lactone is greatly favored at alkaline pH.



3.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
 <i>Information Note: Additional information about the current topic or procedure.</i>	
 Important Note: Information critical to the success of the current procedure or use of the product.	
   etc.	Stages in a process that usually occur in the order listed.
   etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Trademarks

All product names and trademarks are the property of their respective owners.

4.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

