

TECHNICAL INFORMATION

Catalog Number: 100168, 159859, 194121

Aldolase

Molecular Weight: 161,000 ± 3,000 with subunits having a molecular weight of 40,000.^{14,15}

CAS #: 9024-52-6

Synonym: D-Fructose-1,6-bisphosphate-D-glyceraldehyde-3-phosphate lyase

E.C. 4.1.2.13

Source: *Rabbit muscle*

Unit Definition: One unit is the change in absorbancy of 1.00 per minute at 25°C, pH 7.5

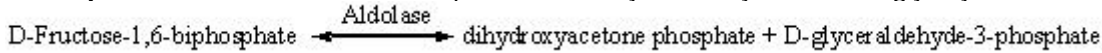
Optimum pH: 7.0

Isoelectric Point: pH 6.1 (0.1 M Phosphate buffer)

Inhibitors: Aldolase is inhibited by Cu²⁺, Ag⁺, Zn²⁺ and o-phenanthroline.⁸ It is inactivated by N-bromoacetyethanolamine phosphate⁵ and pyridoxal phosphate.⁴

Stability: Aldolase is irreversibly denatured at pH values less than 4.5.¹³

Description: Aldolase is a tetrameric protein. It catalyzes a key reaction in glycolysis and energy production:



Aldolase is present in all animal tissue and in most microorganisms. There are two classes of aldolases. Class I aldolase is found in animal and higher plant tissue. Class II aldolase is found in primitive cells such as yeasts and bacteria.

Class I aldolase is characterized by not requiring a bivalent metal cofactor and the formation of a ketimine Schiff base intermediate with the substrate dihydroxyacetone phosphate.

Class II aldolase requires a metal cofactor and is inhibited by EDTA.

Three types of aldolase exist in animal tissue. The major form, type A is found in muscle; type B is found in liver tissue and type C (plus some type A) is found in brain tissue. Aldolase forms five isozymes which may to various degrees be organ specific.

Typical Assay:

Based on Boyer's modification of the hydrazine assay⁷ in which 3-phosphoglyceraldehyde reacts with hydrazine to form a hydrazone which absorbs at 240 nm.

Reagents:

A. 0.0001 M EDTA, pH 7.5, containing 0.0035 M hydrazine sulfate.

B. Substrate: 0.012 M Fructose-1,6-bisphosphate, pH 7.5

Enzyme:

Immediately before use, dilute in water to a concentration of 0.5-2.0 units per ml.

$$\text{mg/ml} = A_{280} \times 1.1$$

Procedure:

Set spectrophotometer at 240 nm and 25°C.

Pipette into cuvettes as follows:

	Blank	Test
Reagent B	--	1.0 ml
Reagent A	2.0 ml	2.0 ml
Distilled water	1.0 ml	--

Record DA₂₄₀ for 10 minutes.

Add:

Enzyme Solution	--	0.1 ml
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Subtract DA₂₄₀/min of the blank from DA₂₄₀/min of the test.

Calculation:

$$\Delta A_{240}/\text{min}$$

$$\text{Unit/mg} = \frac{\Delta A_{240}/\text{min}}{\text{mg enzyme/ml reaction mixture}}$$

Availability:

Catalog Number	Description	Size
100168	Aldolase, crystalline suspension in an ammonium sulfate solution; activity approximately 15-20 units/mg protein	10 mg 20 mg 100 mg 500 mg 1 g
194121	Aldolase, 2X crystallized, supplied as a suspension in an ammonium sulfate solution; Activity not less than 10 units/mg protein	5 mg 25 mg 100 mg
159859	Aldolase, lyophilized powder; activity not less than 10 units/mg protein	25 mg 100 mg

References:

- Anderson, P., Gibbons, I. and Perham, R., "A comparative study of the structure of muscle fructose 1,6-diphosphate aldolases." *Eur. J. Biochem.*, v. **11**, 503 (1969).
- Crowder, A, Barker, R. and Swenson, C., "Ultraviolet difference spectroscopic studies of the binding of ligands to rabbit muscle aldolase." *Biochem.*, v. **12**, 2078 (1973).
- Crowder, A., Swenson, C. and Barker, R., "Colorimetric studies of the binding of ligands to aldolase." *Biochem.*, v. **12**, 2852 (1973).
- Davis, L., Ribereau-Gayon, G and Horecker, B., "Photoinactivation of aldolases by pyridoxal phosphate and its analogues." *Proc. Natl. Acad. Sci. USA*, v. **68**, 416 (1971).
- Hartman, F., Suh, B., Welch, M and Barker, R., "Inactivation of class I fructose diphosphate aldolases by the substrate analog N-bromacetyethanolamine phosphate." *J. Biol. Chem.*, v. **248**, 8233 (1973).
- Heron, E. and Capriolo, R., "Classification of Fructose-1,6-biphosphate aldolases based on ¹⁸O retention in the cleavage reaction." *Biochim. Biophys. Acta*, v. **403**, 563 (1975).
- Jagannathan, V., Sing, K. and Damodaran, M., "Carbohydrate metabolism in citric acid fermentation. IV. Purification and properties of aldolase from *Aspergillus niger*." *Biochem. J.*, v. **63**, 94 (1956).
- Kobashi, K and Horecker, B., "Reversible inactivation of rabbit muscle aldolase by o-Phenanthroline." *Arch. Biochem. Biophys.*, v. **121**, 178 (1967).
- Leberherz, H. and Rutter, W., "Distribution of fructose diphosphate aldolase variants in biological systems." *Biochem.*, v. **8**, 109 (1969).
- Leberherz, H., Bradshaw, R. and Rutter, W., "Structural comparisons between the class I fructose diphosphate aldolases from *Micrococcus aerogenes* and Rabbit." *J. Biol. Chem.*, v. **248**, 1660 (1973).
- London, J., "Variations in the quaternary structure of three lactic acid bacteria aldolases. Evidence for the existence of a class I and class II aldolase in *Lactobacillus casei*." *J. Biol. Chem.*, v. **249**, 7977 (1974).
- Penhoet, E., Kochman, M., Valentine, R. and Rutter, W., "The subunit structure of mammalian fructose diphosphate aldolase." *Biochem.*, v. **6**, 2940 (1967).
- Rutter, W., "Aldolase" in *The Enzymes*, v, **2nd ed.**, (Boyer, P., Lardy, H. and Myrback, K. eds), Academic Press, NY, p. 341 (1961).
- Sia, C. and Horecker, B., "The molecular weight of rabbit muscle aldolase and the properties of the subunits in acid solution." *Arch. Biochem. Biophys.*, v. **123**, 186 (1968).
- Szuchet, S. and Yphantis, D., "Equilibrium sedimentation of proteins in acid solutions. Dissociation of aldolase by aqueous acetic acid." *Biochem.*, v. **12**, 5115 (1973).