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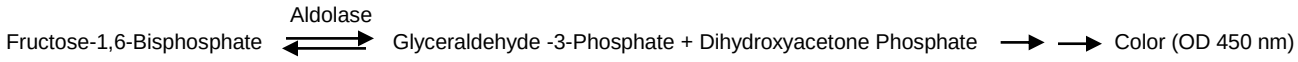
Aldolase Activity Colorimetric Assay Kit

(Catalog # K665-100; 100 assays; Store at -20°C) *4/14*

I. Introduction:

Aldolase (Fructose-Bisphosphate Aldolase: EC 4.1.2.13) is an important enzyme for both glycolysis and gluconeogenesis. It catalyzes the reversible reaction of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate & dihydroxyacetone. There are 2 classes of Aldolase - class I: found in animal and plant tissues and class II: found in prokaryotes and lower eukaryotes. Class I Aldolase has 3 isozymes- Type A: found in muscle and red blood cells, Type B: found in liver and kidney and Type C: found in brain. Aldolase A deficiency leads to myopathy & hemolytic anemia. Muscle disease and liver injury can also cause increased serum aldolase. Accurate detection of aldolase activity is valuable for diagnostic and mechanistic studies. In BioVision's Aldolase Activity Assay, aldolase converts fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone, and through a series of reactions, reduces a nearly colorless probe to a colored product with absorbance at 450 nm. This assay kit is simple, sensitive, and high-throughput adaptable. Detection limit: less than 0.1 mU of aldolase activity in a variety of samples.



II. Applications:

- Measurement of Aldolase activity in various tissues/cells
- Analysis of glycolysis and gluconeogenesis pathways

III. Sample Type:

- Animal tissues: muscle, liver, heart, kidney, etc.
- Cell culture: adherent or suspension cells
- Human serum and plasma

IV. Kit Contents:

Components	K665-100	Cap Code	Part Number
Aldolase Assay Buffer	25 ml	WM	K665-100-1
Aldolase Substrate (Lyophilized)	1 vial	Blue	K665-100-2
Aldolase Enzyme Mix (Lyophilized)	1 vial	Green	K665-100-3
Aldolase Developer (Lyophilized)	1 vial	Red	K665-100-4
NADH Standard (Lyophilized)	1 vial	Yellow	K665-100-5
Aldolase Positive Control (Lyophilized)	1 vial	Orange	K665-100-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20ºC, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **Aldolase Assay Buffer:** Bring to room temperature before use. Store at 4ºC or -20°C.
- **Aldolase Substrate:** Reconstitute with 220 µl dH2O. Pipette up and down to dissolve. Store at -20°C. Use within two months.
- **Aldolase Enzyme Mix:** Reconstitute with 220 µl Assay Buffer. Aliquot and store at -70°C. Keep on ice while in use. Use within two months.
- **Aldolase Developer:** Reconstitute with 220 µl dH_2O. Pipette up and down to dissolve. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 400 µl dH_2O to generate 1.25 mM NADH Standard solution. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.
- **Aldolase Positive Control:** Reconstitute with 200 µl dH_2O and mix thoroughly. Aliquot and store at –70°C. Keep on ice while in use. Use within two months.

VII. Aldolase Activity Assay Protocol:

1. **Sample Preparation:** Serum or plasma samples can be measured directly. For cells or tissues, rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold Aldolase Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 g for 5 min. and collect the supernatant. Add 1-50 µl sample into desired well in a 96-well plate & adjust the volume to 50 µl with Aldolase Assay Buffer. For Aldolase Positive Control, add 2-20 µl Aldolase Positive Control into desired well(s) and adjust the volume to 50 µl with Aldolase Assay Buffer.

   Note:

   a. For unknown samples, we suggest doing pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples having background, prepare parallel sample well(s) as sample background control(s).

2. **NADH Standard Curve:** Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard into a series of wells in 96-well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well NADH Standard. Adjust the volume to 50 µl/well with Aldolase Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix*
Aldolase Assay Buffer	44 µl	46 µl
Aldolase Enzyme Mix	2 µl	2 µl
Aldolase Developer	2 µl	2 µl
Aldolase Substrate	2 µl	----

Add 50 µl of Reaction Mix to each well containing Standards, Positive Control and test samples. Mix well.

*For samples having high background, add 50 µl of Background Control mix to sample background control well(s) and mix.

4. **Measurement:** Measure absorbance (450 nm) immediately in kinetic mode for 10-60 min. at 37°C.

Note: Incubation time depends on the Aldolase activity in the samples. We recommend measuring absorbance in kinetic mode and choosing two time points (T_1 & T_2) in the linear range to calculate the Aldolase activity of the samples. The NADH Standard Curve can be read in Endpoint mode (i.e. at the end of incubation time).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADH Standard Curve. If sample background control reading is significant, subtract sample background control reading from sample reading. Calculate the Aldolase activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated by Aldolase in samples during the reaction time ($\Delta T = T_2 - T_1$).

Sample Aldolase Activity = B/(ΔT x V) x D = nmol/min/µl = mU/µl = U/ml

Where: **B** is NADH amount from Standard Curve (nmol).
Δ**T** is reaction time (min.)
V is sample volume added into the reaction well (µl).
D is dilution factor

Sample Aldolase activity can also be expressed in U/mg tissue or protein.

Unit Definition: One unit of Aldolase is the amount of enzyme that generates 1.0 µmol of NADH per min. at pH 7.2 at 37°C.

(a) (b) (c)

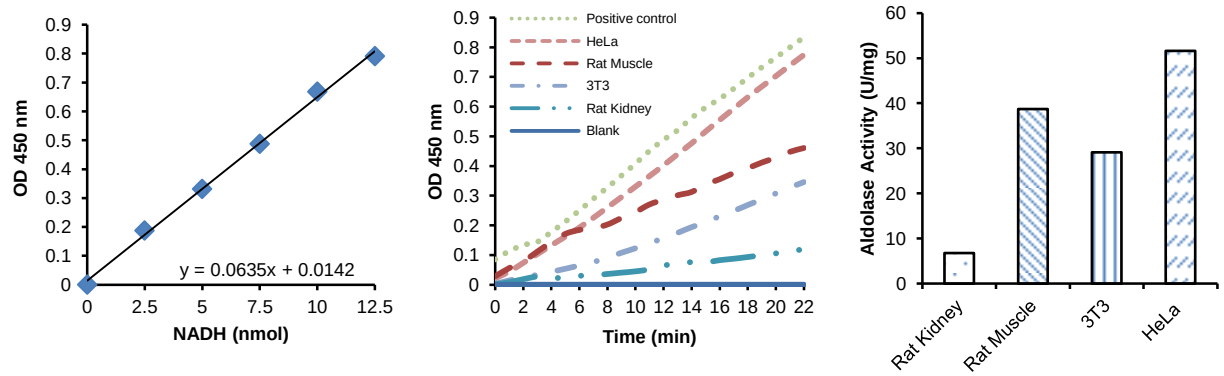


Figure: (a) NADH Standard Curve, (b) Aldolase Activity in rat kidney & muscle & HeLa & 3T3 cells. (c) Relative Aldolase Activity in lysates prepared from rat kidney (14.2 µg), rat muscle (10.64 µg), 3T3 cells (9.97 µg), and HeLa cells (11.35 µg). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Glucose Colorimetric Assay kit II (K686)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
Glucose-3-Phosphate Colorimetric Assay Kit (K641)
PEP Colorimetric/Fluorometric Assay Kit (K365)
Phosphoglucomutase Colorimetric Assay Kit (K774)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)
Glucose Uptake Colorimetric Assay Kit (K676)
Glucose Colorimetric/Fluorometric Assay kit (K606)
2-Phosphoglycerate Colorimetric/Fluorometric Assay Kit (K778)
Hexokinase Colorimetric Assay Kit (K789)
Phosphofructokinase (PFK) Activity Colorimetric Assay Kit (K776)
Phosphoglucose Isomerase Colorimetric Assay Kit (K775)
Pyruvate Kinase Activity Colorimetric/Fluorometric Assay Kit (K709)
Glucose Uptake Fluorometric Assay Kit (K666)

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