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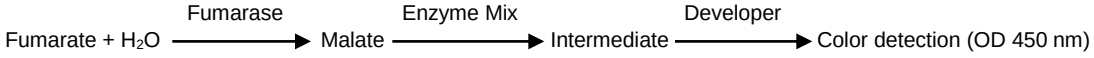
Fumarase Activity Colorimetric Assay Kit

03/14

(Catalog # K596-100; 100 assays; Store at -20°C)

I. Introduction:

Fumarase (Fumarate Hydratase: EC 4.2.1.2) is an enzyme that catalyzes the reversible reaction of hydration and dehydration of fumarate to Malate. It has two forms: mitochondrial and cytosolic forms. The mitochondrial form of fumarase is one of the key enzymes in citric acid cycle, while cytosolic form is important for metabolism of amino acids and fumarate. In humans, fumarase deficiency leads to serious health problems such as fetal brain abnormality, hypotonia, and renal cell carcinoma. Therefore, accurate measurement of fumarase activity is important for preventing, diagnosis and mechanistic study of fumarase deficiency. Biovision's Fumarase Activity Assay kit provides a quick and easy method for monitoring fumarase activity in various samples. In this kit, fumarase converts fumarate into malate, which then reacts with Enzyme Mix to form an intermediate. The intermediate subsequently reduces the Developer to a colored product with strong absorbance at 450 nm. The assay is simple and high-throughput ready and can detect less than 50 U/ml of fumarase activity in variety of sample types.



II. Application:

- Measurement of fumarase activity in various tissues/cells.
- Analysis of citric acid cycle, reductive carboxylation cycle (CO_2 fixation) and renal cell carcinomas.

III. Sample Type:

- Animal tissues: liver, heart, muscle etc.
- Isolated mitochondria.
- Cell culture: Adherent or suspension cells.

IV. Kit Contents:

Components	K596-100	Cap Code	Part Number
Fumarase Assay Buffer	22 ml	WM	K596-100-1
Fumarase Substrate	0.2 ml	Blue	K596-100-2
Fumarase Enzyme Mix (Lyophilized)	1 vial	Green	K596-100-3
Fumarase Developer (Lyophilized)	1 vial	Red	K596-100-4
NADH Standard (Lyophilized)	1 vial	Yellow	K596-100-5
Fumarase Positive Control (Lyophilized)	1 vial	Orange	K596-100-6

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 -20°C, protected from light. Read the entire protocol before performing the assay.

- **Fumarase Assay Buffer:** Warm to room temperature before use. Store at either 4ºC or -20ºC.
- **Fumarase Substrate:** Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **Fumarase Enzyme Mix:** Reconstitute with 220 µl Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **Fumarase Developer:** Reconstitute with 1.05 ml dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 400 µl dH_2O to generate 1.25 mM (1.25 nmol/µl) NADH Standard solution. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **Fumarase Positive Control:** Reconstitute with 200 µl dH_2O and mix thoroughly. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.

VII. Fumarase Activity Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (~10 mg) or cells (1 x 10^6) with 100 µl ice cold Fumarase Assay Buffer, and incubate on ice for 10 min. Centrifuge at 10,000 X g for 5 min. and collect the supernatant. For fumarase activity in mitochondria, isolate mitochondria from fresh tissues or cells using Biovision's Mitochondrial Isolation Kit for Tissue and Cultured Cells (K288). Add 1-50 µl sample per well and adjust the volume to 50 µl/well with Fumarase Assay Buffer. For Positive Control, add 1-10 µl of Fumarase Positive Control into desired well(s) and adjust the volume to 50 µl with Fumarase 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 of NADH Standard. Adjust the volume to 50 µl/well with Fumarase Assay Buffer.

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

	Reaction Mix	Background Control Mix*
Fumarase Assay Buffer	36 µl	38 µl
Fumarase Enzyme Mix	2 µl	2 µl
Fumarase Developer	10 µl	10 µl
Fumarase Substrate	2 µl	----

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

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

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

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

5. **Calculation:** Subtract 0 NADH Standard reading from all readings. Plot the NADH Standard Curve. For samples having background, correct sample background by subtracting the value derived from the sample background control from sample readings. Calculate the Fumarase activity of the test samples: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated by Fumarase during the reaction time ($\Delta T = T_2 - T_1$).

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

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

Unit Definition: One unit of Fumarase is the amount of enzyme that will generate 1.0 µmol of NADH per min. at pH 9.5 at 37°C.

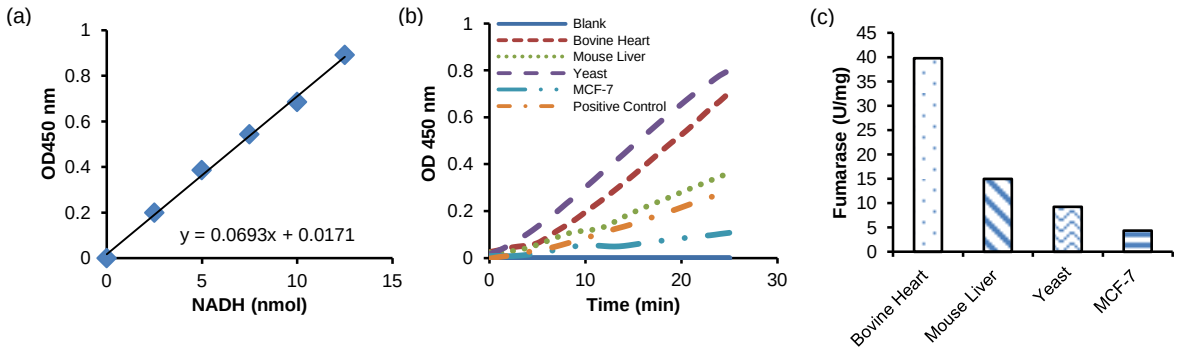


Figure: NADH Standard Curve (a). Fumarase activity in mitochondria isolated from different sources (b). Relative Fumarase activity was calculated in mitochondria prepared from bovine heart (13.75 µg), mouse liver (17.62 µg), yeast (*S. cerevisiae)* (37.8 µg), and MCF-7 cells (16.8 µg) (c). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Fumarate Colorimetric Assay Kit (K633)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
Pyruvate Dehydrogenase (PDH) Activity Colorimetric Assay Kit (K679)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
Succinate Dehydrogenase Activity Colorimetric Assay Kit (K660)
Alpha-Ketoglutarate Colorimetric/Fluorometric Assay Kit (K677)
Mitochondrial Isolation Kit for Tissue and Cultured Cells (K288)
Malate Colorimetric Assay Kit (K637)
Malate Dehydrogenase Activity Colorimetric Assay Kit (K645)
Succinyl CoA Synthetase Activity Colorimetric Assay kit (K597)
Isocitrate Colorimetric Assay Kit (K656)
Isocitrate Dehydrogenase Activity Colorimetric Assay Kit (K756)
Aconitase Activity Colorimetric Assay Kit (K716)

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