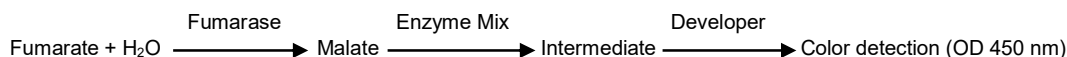


# Fumarase Activity Colorimetric Assay Kit (BN00823)

(Catalog # BN00823; 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. Assay Genie'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<sub>2</sub> 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	BN00823	Cap Code	Part Number
Fumarase Assay Buffer	22 ml	WM	BN00823-1
Fumarase Substrate	0.2 ml	Blue	BN00823-2
Fumarase Enzyme Mix (Lyophilized)	1 vial	Green	BN00823-3
Fumarase Developer (Lyophilized)	1 vial	Red	BN00823-4
NADH Standard (Lyophilized)	1 vial	Yellow	BN00823-5
Fumarase Positive Control (Lyophilized)	1 vial	Orange	BN00823-6

## 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 4°C or -20°C.
- **Fumarase Substrate:** Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **Fumarase Enzyme Mix:** Reconstitute with 220  $\mu$ l 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<sub>2</sub>O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **NADH Standard:** Reconstitute with 400  $\mu$ l dH<sub>2</sub>O to generate 1.25 mM (1.25 nmol/ $\mu$ l) 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  $\mu$ l dH<sub>2</sub>O 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 ( $1 \times 10^6$ ) with 100  $\mu$ l 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 Assay Genie's Mitochondrial Isolation Kit for Tissue and Cultured Cells. Add 1-50  $\mu$ l sample per well and adjust the volume to 50  $\mu$ l/well with Fumarase Assay Buffer. For Positive Control, add 1-10  $\mu$ l of Fumarase Positive Control into desired well(s) and adjust the volume to 50  $\mu$ l 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  $\mu$ l 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  $\mu$ l/well with Fumarase Assay Buffer.
  3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50  $\mu$ l Reaction Mix containing:

	Reaction Mix	Background Control Mix*
Fumarase Assay Buffer	36 $\mu$ l	38 $\mu$ l
Fumarase Enzyme Mix	2 $\mu$ l	2 $\mu$ l
Fumarase Developer	10 $\mu$ l	10 $\mu$ l
Fumarase Substrate	2 $\mu$ l	----

Add 50  $\mu$ l of the Reaction Mix to each well containing the Standard, Positive Control and samples. Mix well.

\*For samples having high background, add 50  $\mu$ l 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  $\Delta OD$  to the NADH Standard Curve to get B nmol of NADH generated by Fumarase during the reaction time ( $\Delta T = T_2 - T_1$ ).

$$\text{Sample Fumarase Activity} = B / (\Delta T \times V) \times D = \text{nmol/min}/\mu\text{l} = \text{mU}/\mu\text{l} = \text{U/ml}$$

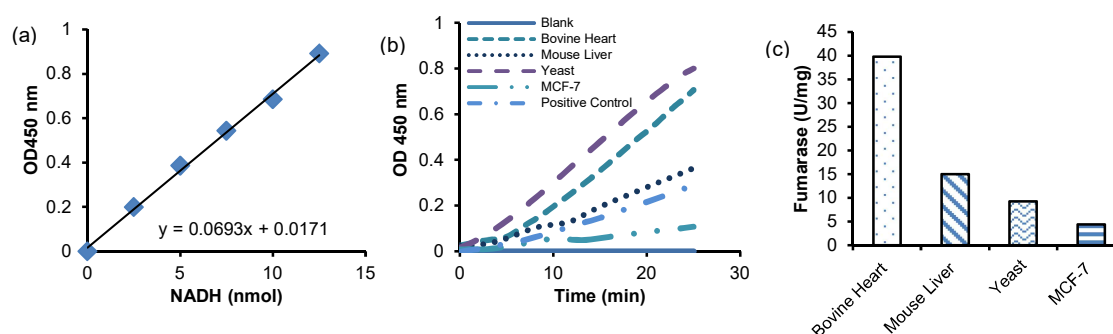
Where: **B** is NADH amount in the sample well from Standard Curve (nmol)

$\Delta T$  is reaction time (min.)

**V** is sample volume added into the reaction well ( $\mu$ l)

**D** is sample dilution factor

**Unit Definition:** One unit of Fumarase is the amount of enzyme that will generate 1.0  $\mu$ mol 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  $\mu$ g), mouse liver (17.62  $\mu$ g), yeast (*S. cerevisiae*) (37.8  $\mu$ g), and MCF-7 cells (16.8  $\mu$ g) (c). Assays were performed following the kit protocol.

**FOR RESEARCH USE ONLY! Not to be used on humans.**