

Fumarate Assay Kit (Colorimetric)

LS-K215-100 (100 Tests) • Store at -20°C



Introduction

FUMARATE, or Fumaric Acid, is one of the key components in TCA cycle and is used by cells to form ATP. Human skin when exposed to sunlight will naturally produce fumaric acid. Fumarate is used as an additive by the food and beverage industries. Fumaric acid esters are also used to treat psoriasis. Increased urinary fumarate may be due to impaired Krebs cycle function, a defect in the enzyme fumarase or mitochondrial function.

LSBio's fumarate assay kit is based on fumarase catalyzed hydration of fumarate to malate. The malate is then oxidized by malate dehydrogenase generating NADH which reduces a formazan (MTT) dye. The intensity of the product color, measured at 565 nm is proportional to the fumarate concentration in the sample.

Key Features

- Fast and sensitive. Use of 20 µL sample. Linear detection range 0.005 to 2 mM fumarate in 96-well plate assay.
- Convenient. The procedure involves adding a single working reagent, and reading the optical density after 30 minutes. Room temperature assay. No 37°C heater is needed.
- High-throughput. Can be readily automated as a high-throughput 96- well plate assay for thousands of samples per day.

Applications

- Direct Assays: fumarate in food, beverage and other biological samples (e.g. cell lysate, tissue homogenate, serum)

Components

Component	K215-100
	100 Tests
Assay Buffer	10 mL
NAD/MTT	1 mL
Standard	1 mL
Enzyme A	120 µL
Enzyme B	120 µL
FMR Enzyme	120 µL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipette), clear flat bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader capable of reading absorbance between 520-600 nm.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

FOR RESEARCH USE ONLY! Not for use in humans.

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Assay Procedure

Solid samples (food, fruits etc) can be homogenized in water followed by filtration or centrifugation (e.g. 5 min 14,000 rpm). Beverage samples can be assayed directly. Check the pH of the sample. If necessary, adjust the sample pH to 7-8 with NaOH or HCl. Samples containing carbon dioxide should be degassed by gentle stirring prior assay.

Tissue: Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 μ L buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

It is prudent to test several dilutions to determine an optimal dilution factor n.

Procedure using 96-well plate

1. Standards. Prepare 200 μ L 2 mM Premix by mixing 20 μ L of the Standard (20 mM) and 180 μ L distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H ₂ O	Fumarate (mM)
1	200 μ L + 0 μ L	2.0
2	60 μ L + 40 μ L	1.2
3	30 μ L + 70 μ L	0.6
4	0 μ L + 100 μ L	0

Transfer 20 μ L standards into separate wells of a clear, flat-bottom 96- well plate.

2. Transfer 20 μ L of each sample into separate wells.

If a sample known to contain malate, a sample blank will be needed. Transfer 20 μ L of the sample into two separate wells.

3. Prepare enough Working Reagent (WR) for sample and standard wells by mixing, for each well: 74 μ L Assay Buffer, 8 μ L NAD/MTT Solution, 1 μ L Enzyme A, 1 μ L Enzyme B, and 1 μ L FMR Enzyme. Fresh reconstitution of the WR is recommended.

If Sample Blanks are needed, prepare enough Blank Working Reagent (BWR) for the sample blank wells by mixing, for each well: 75 μ L Assay Buffer, 8 μ L NAD/MTT Solution, 1 μ L Enzyme A, and 1 μ L Enzyme B (i.e. NO FMR ENZYME).

4. Add 80 μ L WR or BWR to the corresponding wells. Tap plate briefly to mix.
5. Incubate at room temperature for 30 min. Read OD565nm (520-600 nm).

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Calculations

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the fumarate concentration of Sample,

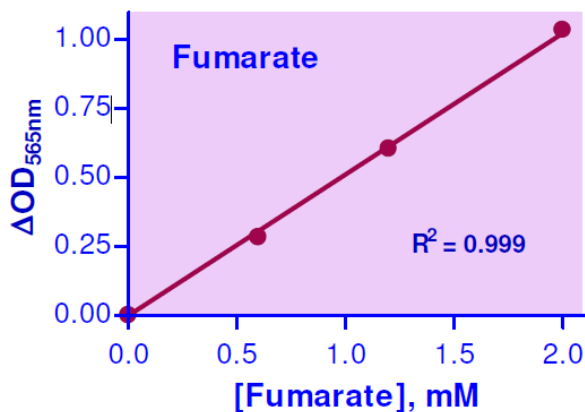
$$[\text{Fumarate}] = \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{\text{Slope (mM}^{-1})} \times n \text{ (mM)}$$

OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Sample Blank, respectively. n is the sample dilution factor.

Note: if the sample OD value is higher than OD for 2 mM fumarate standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM fumarate equals 11.6 mg/dL, or 116 ppm.

Sample Data



Standard Curve in 96-well plate assay in water.

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