EnzyChrom™ Fumarate Assay Kit (EFUM-100)

Quantitative Colorimetric Fumarate (Fumaric Acid) Determination

DESCRIPTION

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.

BioAssay Systems' 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)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Enzyme A:**	120 µL
NAD/MTT:	1 mL	**Enzyme B:**	120 µL
Standard:	1 mL 20 mM Fumarate	**FMR Enzyme:**	120 µL

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation

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. *S*amples 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 µL 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 µL 2 mM Premix by mixing 20 µL of the Standard (20 mM) and 180 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H_2O	Fumarate (mM)
1	200 µL + 0 µL	2.0
2	60 µL + 40 µL	1.2
3	30 µL + 70 µL	0.6
4	0 µL + 100 µL	0

   Transfer 20 µL standards into separate wells of a clear, flat-bottom 96-well plate.
2. Transfer 20 µL of each sample into separate wells.

   If a sample known to contain malate, a sample blank will be needed. Transfer 20 µL of the sample into two separate wells.
3. Prepare enough Working Reagent (WR) for sample and standard wells by mixing, for each well: 74 µL Assay Buffer, 8 µL NAD/MTT Solution, 1 µL Enzyme A, 1 µL Enzyme B, and 1 µL 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 µL Assay Buffer, 8 µL NAD/MTT Solution, 1 µL Enzyme A, and 1 µL Enzyme B (i.e. NO FMR ENZYME).
4. Add 80 µL WR or BWR to the corresponding wells. Tap plate briefly to mix.
5. Incubate at room temperature for 30 min. Read OD_{565nm} (520-600 nm).

CALCULATION

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,

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

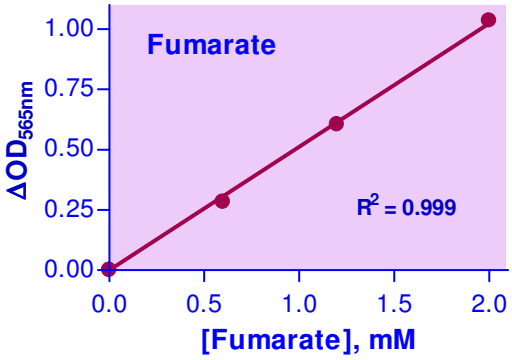
OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), 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.



Standard Curve in 96-well plate assay in water.

LITERATURE

1. M, Ameen and R., Russell (1999). Fumaric acid esters: an alternative systemic treatment for psoriasis. Clin Exp Dermatol. 24(5): 361-4.
2. Aquilina, G et al (2013). Scientific opinion on the safety and efficacy of fumaric acid as a feed additive for all animal species. EFSA Journal. 11(2): 3102.
3. Ali, M. (2003). The Cause of Fibromyalgia: the respiratory-to fermentative shift (the DysOx State) in ATP production, J. Integrative Medicine. 8: 135-140.