EnzyChrom™ Fumarase Assay Kit (EFMR-100)

Quantitative Colorimetric Kinetic Fumarase Activity Determination

DESCRIPTION

FUMARASE (OR FUMARATE HYDRATASE) (EC 4.2.1.2) is an enzyme that catalyzes the reversible hydration/dehydration reaction of fumarate to malate. Fumarase exists in two isoforms: a cystosolic and mitochondrial form. In the citric acid cycle, it facilitates a transition step in the production of energy in the form of NADH. Fumarase deficiency in humans results in early brain development problems and is characterized by poor feeding, hypotonia, failure to thrive, etc.

BioAssay Systems' non-radioactive, colorimetric fumarase assay is based on the reduction of the tetrazolium salt MTT in a NADH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is proportional to the enzyme activity.

KEY FEATURES

Fast and sensitive. Linear detection range: 0.4 to 70 U/L for 30 min reaction at 37˚C.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

Fumarase activity determination in biological samples (e.g. plasma, serum, erythrocytes, tissue and culture media.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Enzyme A:**	120 µL
NAD/MTT:	1 mL	**Enzyme B:**	120 µL
Substrate:	600 µL	**Calibrator:**	1.5 mL

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. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

Sample Preparation: Serum and plasma are assayed directly.

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL cold 50 mM potassium phosphate buffer, pH 7.5. Centrifuge at 14,000 × *g* for 10 min at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells by centrifugation at 2,000 x *g* for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 14,000 × *g* for 10 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation: Equilibrate reagents to desired reaction temperature (37°C is recommended). Keep enzymes on ice during experiment. Briefly centrifuge tubes before use.

Assay Procedure:

1. Transfer 100 µL H_2O (OD_{H2O}) and 100 µL Calibrator (OD_{CAL}) solution into separate wells of a clear flat bottom 96-well plate.
2. Transfer 20 µL H_2O into one well, this will be the blank. Transfer 20 µL of each sample into separate wells.
3. Prepare enough Working Reagent (WR) for all reaction wells by mixing, for each 96-well assay: 75 µL Assay Buffer, 8 µL NAD/MTT, 5 µL Substrate, 1 µL Enzyme A, 1 µL Enzyme B.

   Add 80 µL WR to all samples and blank wells. Tap plate briefly to mix.
4. Read OD_{565nm} at time 10 min (OD_{10}) and time 40 min (OD_{40}) on a plate reader.

CALCULATION

Subtract the OD_{10} from OD_{40} for each sample to compute the ΔOD_S values, do the same for the blank to compute ΔOD_B. Fumarase activity can then be calculated as follows:

$$\text{FUM Activity} = \frac{\Delta OD_S - \Delta OD_B}{\varepsilon_{mtt} \cdot l} \times \frac{\text{Reaction Vol (µL)}}{t\,\text{(min)} \cdot \text{Sample Vol (µL)}} \times n$$

$$= \frac{273}{t\,\text{(min)}} \times \frac{\Delta OD_S - \Delta OD_B}{OD_{CAL} - OD_{H20}} \times n \quad \textit{(U/L)}$$

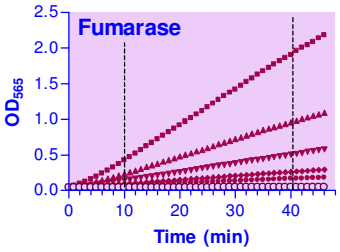
where ε_{mtt} is the molar absorption coefficient of reduced MTT. *l* is the light path length which is calculated from the calibrator. OD_{CAL} and OD_{H20} are OD_{565nm} (OD_{10}) values of the Calibrator and water. *t* is the difference in time between readings (30 min is the recommended time at 37˚C). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. *n* is the dilution factor if the sample needed to be diluted.

Unit definition: 1 Unit (U) of Fumarase will catalyze the conversion of 1 µmole of L-fumarate to L-malate per minute at pH 7.8.

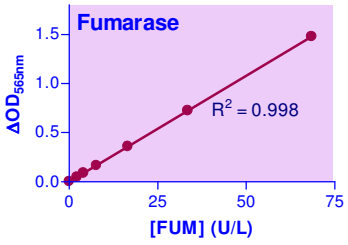
Note: If sample Fumarase activity exceeds 70 U/L, dilute samples in water and repeat the assay. For samples with Fumarase activity < 1 U/L, the incubation time can be extended to 2 hours.

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.



Raw Kinetic Data



FUM Activity (30 min, 37 °C)

LITERATURE

1. Saini, A. and Pratibha S. (2013) Infantile metabolic encephalopathy due to fumarase deficiency. Journal of child neurology 28.4: 535-537.
2. Yogev, O, et al. (2010) Fumarase: a mitochondrial metabolic enzyme and a cytosolic/nuclear component of the DNA damage response. PLoS biology 8.3: e1000328.
3. Rustin, P., et al. (1997) Inborn errors of the Krebs cycle: a group of unusual mitochondrial diseases in human. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1361.2 : 185-197.