Technical Bulletin

Fumarase Assay Kit

Catalogue number MAK521

Product Description

Fumarase (or Fumarate Hydratase) is an enzyme that catalyzes the reversible hydration/dehydration reaction of fumarate to malate. Fumarase exists in two isoforms: a cytosolic and mitochondrial form. In the citric acid cycle, it facilitates a transition step in the production of energy in the form of NADH.

This 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.

The linear detection range of the Fumarase Assay Kit is 0.4 to 70 U/L fumarase. The kit is suitable for determination of fumarase in biological samples such as plasma, serum, erythrocytes, tissue and culture media.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK521A
- Enzyme A 120 µL
  Catalogue Number MAK521B
- Enzyme B 120 µL
  Catalogue Number MAK521C
- NAD/MTT 1 mL
  Catalogue Number MAK521D
- Substrate 600 µL
  Catalogue Number MAK521E
- Calibrator 1.5 mL
  Catalogue Number MAK521F

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader.
- Clear flat-bottom 96-well plates (for example, Corning Costar). Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL centrifuge tubes
- Refrigerated microcentrifuge capable of RCF ≥ 14,000 × g.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate reagents to desired reaction temperature.

Enzyme A and B: Keep enzymes on ice during experiment.

Note: 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 (for example, 25 °C or 37 °C).

Procedure

All samples and standards should be run in duplicates.

Sample Preparation

Serum and plasma samples can be assayed directly.

Tissue Samples:

1. Rinse tissue in phosphate buffered saline (pH 7.4) to remove blood, prior to dissection.
2. Homogenize 50 mg of tissue in ~200 µL cold 50 mM potassium phosphate buffer, pH 7.5.
3. Centrifuge at 14,000 × g for 10 minutes at 4 °C. Remove supernatant for assay.

Cell Samples:

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

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

Assay

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.

Working Reagents

1. Mix enough reagents for the number of assays to be performed. Prepare Working Reagents according to Table 1. For each well, prepare 89 µL of Working Reagent.

Table 1.

Preparation of Working Reagents

Reagent	Working Reagent
Assay Buffer	74 µL
NAD/MTT	8 µL
Substrate	5 µL
Enzyme A	1 µL
Enzyme B	1 µL

2. Add 80 µL of Working Reagent to blank and sample wells and tap gently to mix.

Measurement

1. Read the optical density at 565 nm at 10 minutes and at 40 minutes on a plate reader.

Results

1. Subtract the Optical Density values recorded at 10 mins from 40 min for each sample to compute the ΔOD_S values.
2. Repeat the same for the blank to compute ΔOD_B values.
3. Fumarase activity can be calculated using the below equation:

   Fumerase Activity (U/L) =

$$\frac{\Delta OD_S - \Delta OD_B}{\varepsilon_{mtt} \cdot l} \times \frac{\text{Reaction Vol (µL)}}{t(\text{min}) \cdot \text{Sample Vol (µL)}} \times DF$$

$$= \frac{273}{t\ (\text{min})} \times \frac{\Delta OD_S - \Delta OD_B}{OD_{Cal} - \Delta OD_{H_2O}} \times DF$$

Where:

ε_{mtt} = Molar absorption coefficient of reduced MTT.

l = Path length which is calculated from the calibrator.

OD_{CAL} = Optical Density of Calibrator.

OD_B = Optical Density of Blank.

OD_S = Optical Density of Samples.

t = Difference in time between readings.

DF = Dilution factor if the sample needed to be diluted.

Reaction Vol = 100 µL.

Sample Vol = 20 µL.

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.

Figure 1.

Typical raw kinetic data for fumarase.

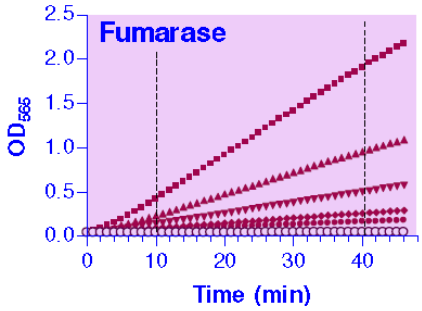
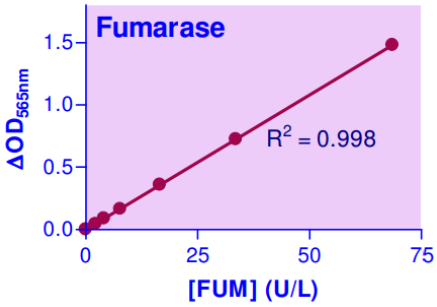


Figure 2.

Representative data of fumarase activity (30 minutes, 37 °C).



Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

mak521pis Rev 09/23