Technical Bulletin

Fumarate Assay Kit

Catalogue Number MAK492

Product Description

Fumarate, or fumaric acid, is one of the key components in the 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 industry. 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 malfunction.

The Fumarate Assay Kit is based on the 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.

The linear detection range of the kit is 0.005 - 2 mM fumarate. The kit is suitable for the determination of fumarate in food, beverage, and other biological samples (for example, cell lysate, tissue homogenate, and serum).

Components

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

- Assay Buffer 10 mL
  Catalogue Number MAK492A
- Enzyme A 120 µL
  Catalogue Number MAK492B
- Enzyme B 120 µL
  Catalogue Number MAK492C
- NAD/MTT 1 mL
  Catalogue Number MAK492D
- FMR Enzyme 120 µL
  Catalogue Number MAK492E
- Standard (20 mM Fumarate) 1 mL
  Catalogue Number MAK492F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL centrifuge tubes
- Dounce tissue grinder set (Catalogue Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥ 14,000 × *g*
- Phosphate Buffered Saline (PBS) (Catalogue Number P3813 or equivalent)
- Potassium phosphate monobasic (Catalogue Number P0662 or equivalent)

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.

Procedure

All samples and standards should be run in duplicates.

Sample Preparation

Solid Samples

Homogenize solid samples such as food or fruits in purified water. Filter or centrifuge at 14,000 × *g* for 5 minutes.

Beverage Samples

Assay can be performed directly on beverage samples. 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 to assay.

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood.
2. Homogenize tissue (50 mg) in ~200 µL of Assay Buffer containing 50 mM potassium phosphate (pH 7.5).
3. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
4. Use the clear supernatant for the assay.

All Samples

Transfer 20 µL of each Sample into separate wells of a 96-well plate. For unknown samples, perform a pilot experiment by testing several dilutions to ensure the readings are within the linear detection range of the kit.

Sample Blank

For samples that contain malate, a sample blank is required. Transfer 20 µL of the Sample into two separate wells, one for the Sample and the second for the Sample Blank.

Standard Curve Preparation

1. Prepare a 2 mM Fumarate Standard by mixing 20 µL of 20 mM Fumarate Standard and 180 µL of purified water.
2. Prepare Fumarate standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Fumarate Standards

Well	2 mM Standard	Purified Water	Fumarate (mM)
1	200 µL	---	2.0
2	60 µL	40 µL	1.2
3	30 µL	70 µL	0.6
4	---	100 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a clear 96-well plate.

Working Reagent

1. Mix enough reagent for the number of assays to be performed. Fresh reconstitution of the Working Reagent is recommended.
   a. For each Sample and Standard well, prepare 85 µL of Working Reagent according to Table 2.
   b. For samples requiring a Sample Blank, prepare 85 µL of Blank Working Reagent for each Sample Blank well according to Table 2.

Table 2.
Preparation of Working Reagent

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	74 µL	75 µL
MTT/NAD	8 µL	8 µL
Enzyme A	1 µL	1 µL
Enzyme B	1 µL	1 µL
FMR Enzyme	1 µL	-

2. Transfer 80 µL of Working Reagent into each Standard and Sample well.
3. Transfer 80 µL of Blank Working Reagent into each Sample Blank well.
4. Tap plate to mix.

Measurement

1. Incubate the plate for 30 minutes at room temperature.
2. Read optical density (OD) at 565 nm.

Note: If the Sample OD value is higher than OD value for the 2.0 mM Fumarate Standard, dilute Sample in purified water and repeat the assay. Multiply the results by the dilution factor (DF).

Results

1. Calculate ΔOD by subtracting the OD reading of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD against standard concentrations and determine the slope of the standard curve.
3. Calculate the fumarate concentration of the Sample:

Fumarate (mM) =

$$\frac{OD_{Sample} - OD_{Blank}}{Slope\ (mM^{-1})}\ x\ DF$$

where:

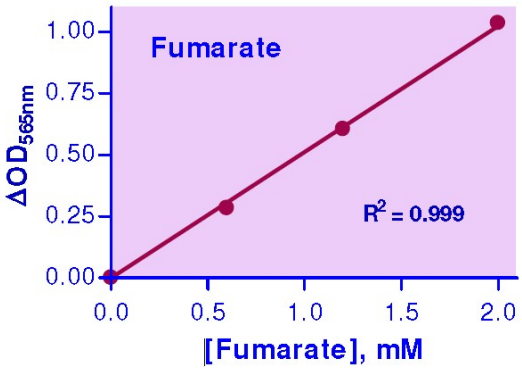
OD_{Sample} = Optical density reading of Sample

OD_{Blank} = Optical density reading of Blank (Standard #4 or Sample Blank)

DF = Sample dilution factor (DF = 1 for undiluted Samples)

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

Typical Fumarate Standard Curve



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.

mak492pis Rev 09/22