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Product Information

Fumarate Assay Kit

Catalog Number **MAK060**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Fumarate is a tricarboxylic acid (TCA) cycle intermediate formed by the oxidation of succinate by succinate dehydrogenase. In the mammalian liver, fumarate is also a product of the urea cycle where its release in the cytosol leads to its conversion into malate and subsequently oxaloacetate while generating NADH in the cytosol. Deficiencies in fumarate hydratase are associated with increased cellular concentrations of fumarate and increased incidences of renal cell cancer. This is most likely due to fumarate-mediated upregulation of HIF1.

The Fumarate Assay Kit provides a convenient tool for sensitive detection of fumarate in a variety of samples. Fumarate is determined by an enzyme assay, which results in a colorimetric (450 nm) product, proportional to the fumarate present.

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

Fumarate Assay Buffer Catalog Number MAK060A	25 mL
Fumarate Enzyme Mix Catalog Number MAK060B	1 vl
Fumarate Developer Catalog Number MAK060C	1 vl
Fumarate Standard, 0.1 M Catalog Number MAK060D	0.2 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Fumarate Assay Buffer – Allow buffer to come to room temperature before use.

Fumarate Enzyme Mix – Reconstitute in 220 µL of Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Fumarate Developer – Reconstitute in 900 µL of water. Mix well by pipetting (don't vortex), then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Fumarate Standards for Colorimetric Detection
Dilute 10 µL of the 0.1 M Fumarate Standard with 990 µL of Fumarate Assay Buffer to generate a 1 mM standard solution. Add 0, 5, 10, 15, 20, and 25 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 5, 10, 15, 20, and 25 nmole/well standards. Add Fumarate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (40 mg) or cells (1×10^6) should be rapidly homogenized in 100 µL of Fumarate Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. Add 1–50 µL samples into duplicate wells of a 96 well plate.

Serum samples (10–50 µL) may be added directly into duplicate wells. Bring samples to a final volume of 50 µL with Fumarate Assay Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 100 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
Fumarate Assay Buffer	90 µL
Fumarate Developer	8 µL
Fumarate Enzyme Mix	2 µL

2. Add 100 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the absorbance at 450 nm (A_{450}).

Results
Calculations
The background for the assay is the value obtained for the 0 (blank) Fumarate standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate Fumarate standards to plot a standard curve. The amount of fumarate present in the samples may be determined from the standard curve.
Note: A new standard curve must be set up each time the assay is run.

Concentration of Fumarate

$$S_a/S_v = C$$

C = Concentration of fumarate in sample
S_a = Amount of fumarate in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added into the wells.

Fumarate molecular weight: 116.07 g/mole

Sample Calculation
Fumarate amount (S_a) = 15.84 nmole
Assay volume (S_v) = 50 µL

Concentration of Fumarate in sample

$$15.84 \text{ nmole}/50 \text{ µL} = 0.316 \text{ nmole/µL}$$

$$0.316 \text{ nmole/µL} \times 116.07 \text{ ng/nmole} = 36.77 \text{ ng/µL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Always prepare fresh Master Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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