



Fumarase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1318

(Version 1.2A)

Detection and Quantification of Fumarase(FUM) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	4
IV. REAGENT PREPARATION.....	5
V. SAMPLE PREPARATION.....	6
VI. ASSAY PROCEDURE.....	7
VII. CALCULATION.....	8
VIII.TYPICAL DATA.....	9

I. INTRODUCTION

Fumarase (EC 4.2.1.2, FUM) is an enzyme that catalyzes the reversible reaction of hydration and dehydration of fumarate to Malate. It has two forms: mitochondrial and cytosolic forms. The mitochondrial form of fumarase is one of the key enzymes in citric acid cycle, while cytosolic form is important for metabolism of amino acids and fumarate. In humans, fumarase deficiency leads to serious health problems such as fetal brain abnormality, hypotonia, and renal cell carcinoma. Therefore, accurate measurement of fumarase activity is important for preventing, diagnosis and mechanistic study of fumarase deficiency.

Fumarase Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring fumarase activity in a variety of samples. This kit is based on fumarase hydrolyzing fumarate, the products generating NADH reduces a formazan reagent. The intensity of the product color in the absorbency at 450nm, resulting from the oxidation of NADH, is a measure of fumarase activity.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	10 mlx 1	4 °C
Substrate	Powderx 1	-20 °C
Enzyme	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20 °C for 3 days after reconstitution. Then dilute to 400 μ mol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5/6.25 μ mol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Keep in dark and store at 4 °C for 1 week or -20 °C for 2 months.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -80 °C and use within 1 month.

Dye Reagent A: Add 9 ml distilled water to dissolve before use, mix. Keep in dark and store at 4 °C for 1 week or -20 °C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 0.1 ml assay buffer to dissolve before use. Aliquot & store at -80 °C and use within 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Reaction Buffer	70 μ l	70 μ l	--	--	70 μ l
Sample	10 μ l	--	--	--	--
Standard	--	--	100 μ l	--	--
Distilled water	--	10 μ l	--	100 μ l	--
Positive Control	--	--	--	--	10 μ l
Substrate	10 μ l	10 μ l	--	--	10 μ l
Enzyme	10 μ l	10 μ l	--	--	10 μ l
Dye Reagent A	90 μ l	90 μ l	90 μ l	90 μ l	90 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l	10 μ l
Mix, keep in dark for 5 minutes at room temperature, record absorbance measured at 450 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of FUM activity is defined as the enzyme converts 1 μmol of NADH per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10⁴

C_{Standard}: the concentration of standard, μmol/mL

V_{Standard}: the volume of standard in assay procedure, mL

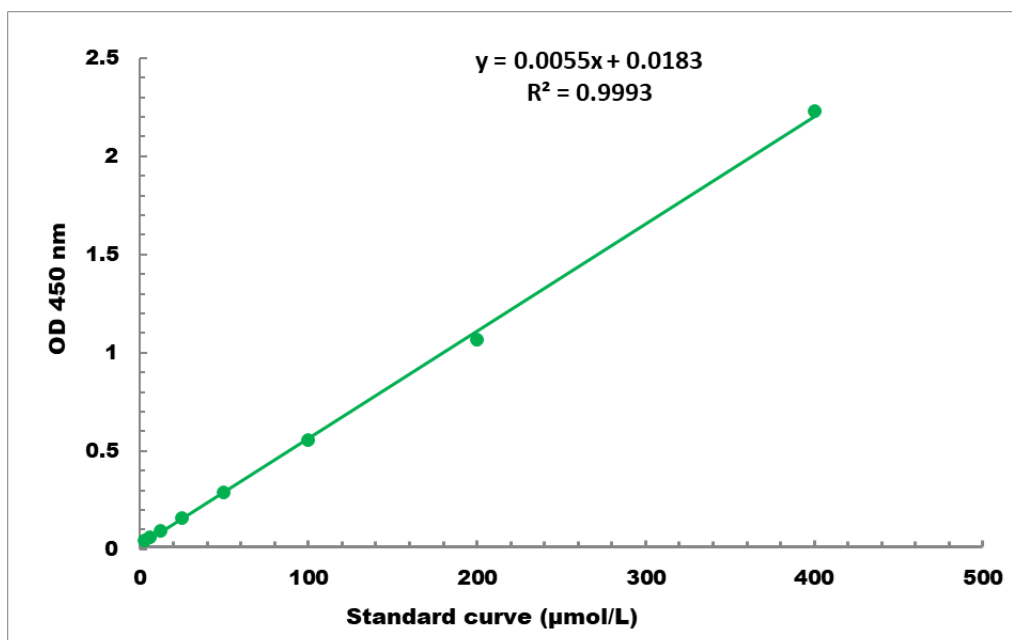
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

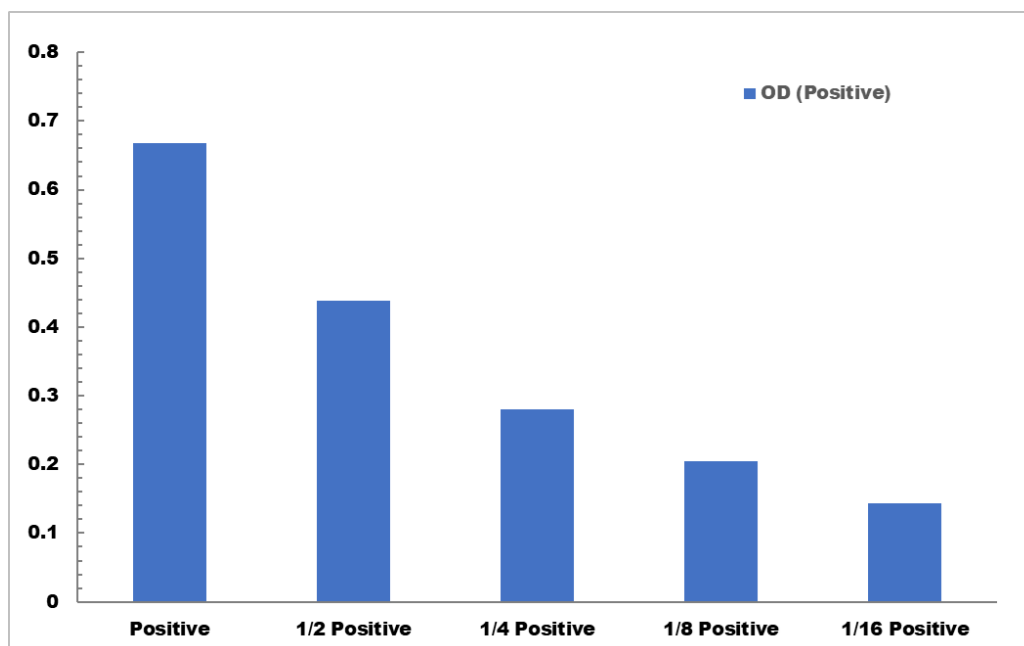
T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 μmol/L - 400 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration