Fumarate Microplate Assay Kit

User Manual

Catalog # CAK1317

(Version 1.1A)

Detection and Quantification of Fumarate content in Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

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

I. INTRODUCTION

Fumarate (HO2CCH=CHCO2H-) is an intermediate in the Kreb's cycle used by cells to metabolize food to form ATP. 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. The human skin naturally produces fumaric acid when exposed to sunlight. In fact, fumaric acid esters have been used to treat psoriasis, possibly due to an impaired production of fumaric acid in the skin. Fumaric acid has also been used in beverages, baking powders and candy.

Fumarate Microplate Assay Kit is designed to directly measure fumarate content in a variety of samples. It is based on fumarase hydrolyzes fumarate. The intermediate subsequently reduces the dye reagent to a colored product with strong absorbance at 450 nm, measured at 450 nm is proportional to the fumarate concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -80 °C. Use within one month.

Dye Reagent A: add 1 ml distilled water to dissolve before use, mix. Store at 4 °C. Use within one month.

Standard: add 1 ml distilled water to dissolve before use, then add 100 µl into 400 µl distilled water, the concentration will be 10 mmol/L. Store at -20 °C. Use within one month.

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

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml distilled water, transfer it into microcentrifuge tube, centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Dye Reagent A	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, keep in dark for 30 minutes at room temperature, record absorbance measured at 450 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$\text{Fumarate (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$\text{Fumarate (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 10 mmol/L = 10 μmol/ml;

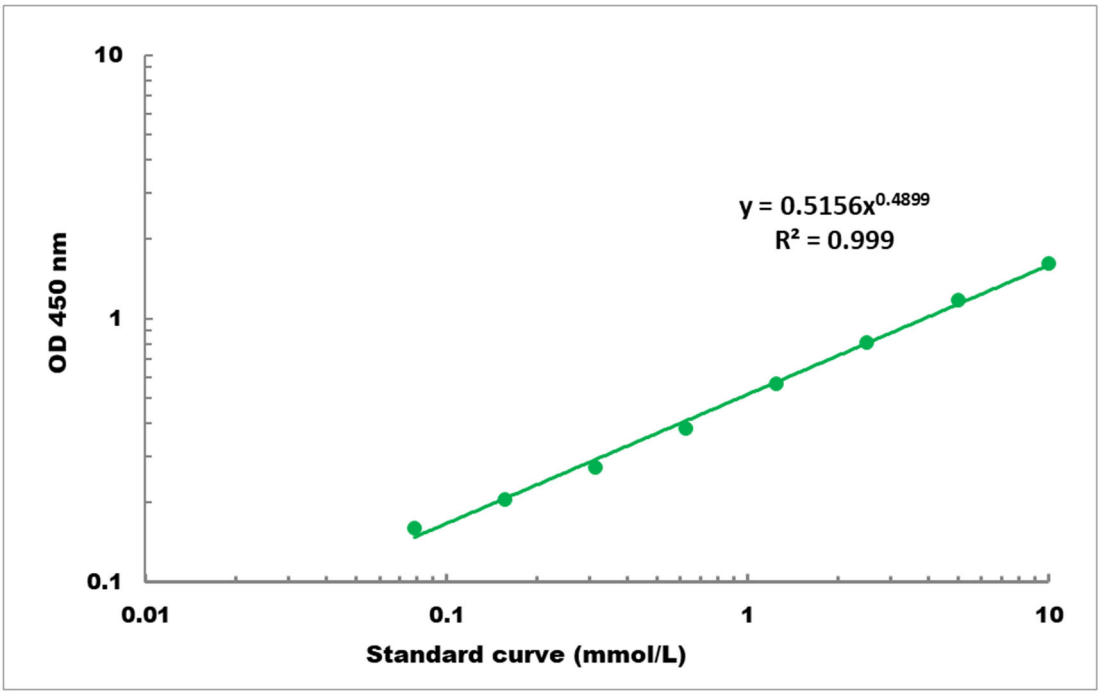
W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 10 μl;

V_{Sample}: the volume of sample, 10 μl.

VII. TYPICAL DATA

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



Detection Range: 0.1 mmol/L - 10 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES