# Fumarase Activity

# Microplate Assay Kit

# User Manual

## Catalog # CAK1318

(Version 1.1A)

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.**

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

## II. KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-Well Microplate | 1 plate | |
| Assay Buffer | 30 ml x 4 | 4 °C |
| Reaction Buffer | 10 ml x 1 | 4 °C |
| Substrate | Powder x 1 | -20 °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 | -20 °C |
| Positive Control | Powder x 1 | -20 °C |
| Technical Manual | 1 Manual | |

**Note:**

**Substrate**: add 1 ml Reaction Buffer to dissolve before use. Store at -20 °C. Use within one month.

**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 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

**Positive Control**: add 0.1 ml assay buffer to dissolve before use. Aliquot & store at -80 °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
8. Ice

## IV. 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 $5 \times 10^6$ 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.

## V. ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents into the microplate:

| Reagent | Sample | Control | Standard | Blank | Positive Control |
|---|---|---|---|---|---|
| Reaction Buffer | 70 µl | 70 µl | -- | -- | 70 µl |
| Sample | 10 µl | -- | -- | -- | -- |
| Standard | -- | -- | 100 µl | -- | -- |
| Distilled water | -- | 10 µl | -- | 100 µl | -- |
| Positive Control | -- | -- | -- | -- | 10 µl |
| Substrate | 10 µl | 10 µl | -- | -- | 10 µl |
| Enzyme | 10 µl | 10 µl | -- | -- | 10 µl |
| Dye Reagent A | 90 µl | 90 µl | 90 µl | 90 µl | 90 µl |
| Dye Reagent B | 10 µl | 10 µl | 10 µl | 10 µl | 10 µl |
| Mix, keep in dark for 5 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) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

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

## VI. CALCULATION

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

1. According to the protein concentration of sample

$$FUM\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$FUM\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$FUM\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$FUM\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 0.4 μmol/ml;

$V_{Standard}$: the volume of standard, 100 μl = 0.1 ml;

$V_{Sample}$: the volume of sample, 0.01 ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

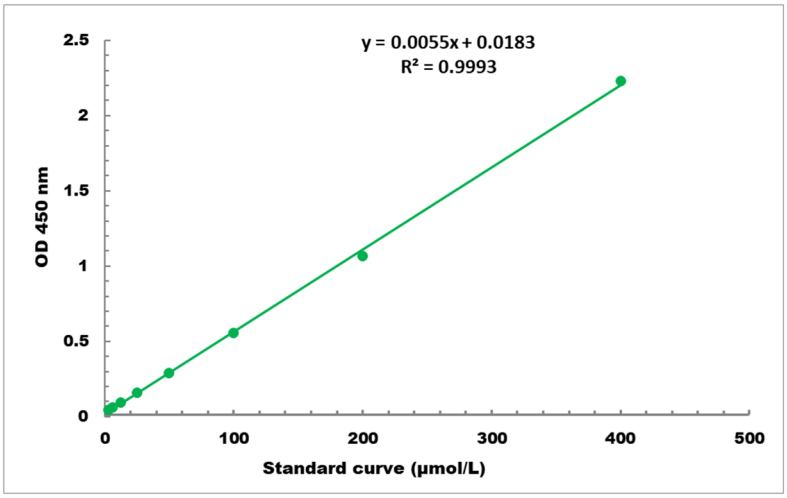
N: the quantity of cell or bacteria, $N \times 10^4$;

$V_{Assay}$: the volume of Assay buffer, 1 ml;

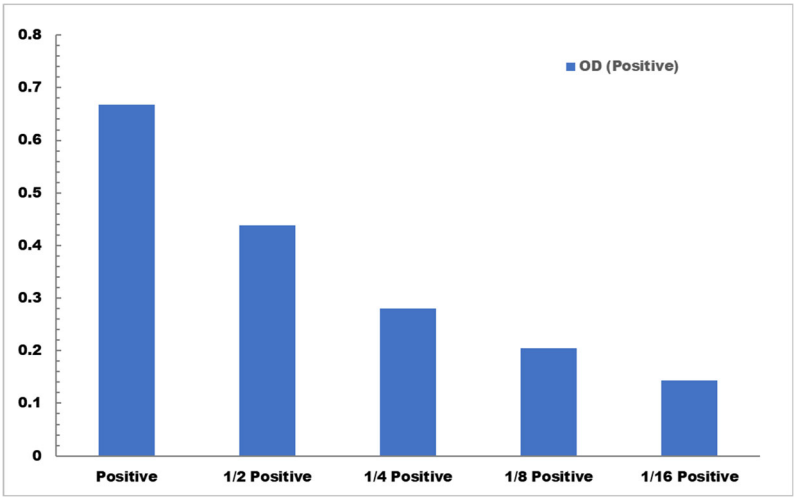
T: the reaction time, 5 minutes.

## VII. 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

## VIII. TECHNICAL SUPPORT

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

## IX. NOTES