Mixed Function Oxidase Microplate Assay Kit User Manual

Catalog # CAK1207

(Version 1.2A)

Detection and Quantification of Mixed Function Oxidase(MFO) Activityin Tissue extracts, Cell lysate and Other biological fluidsSamples.

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

I. INTRODUCTION

Mixed Function Oxidase is the name of a family of oxidase enzymes that catalyze a reaction in which each of the two atoms of oxygen in O_2 is used for a different function in the reaction. Oxidase is a general name for enzymes that catalyze oxidations in which molecular oxygen is the electron acceptor but oxygen atoms do not appear in the oxidized product. Often, oxygen is reduced to either water or hydrogen peroxide. Most of the oxidases are flavoproteins.

Mixed Function Oxidase Microplate Assay Kit is based on the cleavage of 4-nitrophenol from the synthetic substrate. Nitrophenol becomes intensely colored after addition of the stop reagent. The increase in absorbance at 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	-20 °C
Reaction Buffer	10 ml x 1	4 °C
Coenzyme	Powder x 1	-20 °C
Stop Solution	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate:add 3 ml ethanol to dissolve before use.

Coenzyme: add 1 ml Reaction Buffer to dissolve before use.

Standard:add 1 ml Reaction Buffer to dissolve before use, then add 30 µl into 970 µlReaction Buffer, mix; the concentration will be 300 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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

Weighout 0.1 g tissue, homogenize with 1 mlAssay 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 liquidsamples

Detect directly.

V. ASSAY PROCEDURE

Warm all regentsto room temperature before use.

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	100 µl	--
Distilled water	--	--	100 µl
Reaction Buffer	40 µl	--	--
Coenzyme	10 µl	--	--
Substrate	30 µl	--	--
Mix, put it in the oven, 37°C for 30 minutes.			
Stop Solution	100 µl	100 µl	100 µl
Mix, record absorbance measured at 405 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 ofMFO activity is defined as the enzyme generates 1μmol of p-nitrophenol per minute.

1. According to the protein concentration of sample

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

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3.According to the quantity of cells or bacteria

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

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

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

$C_{Standard}$: the concentration of Standard, 300μmol/L = 0.3μmol/ml;

W: the weight of sample, g;

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

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

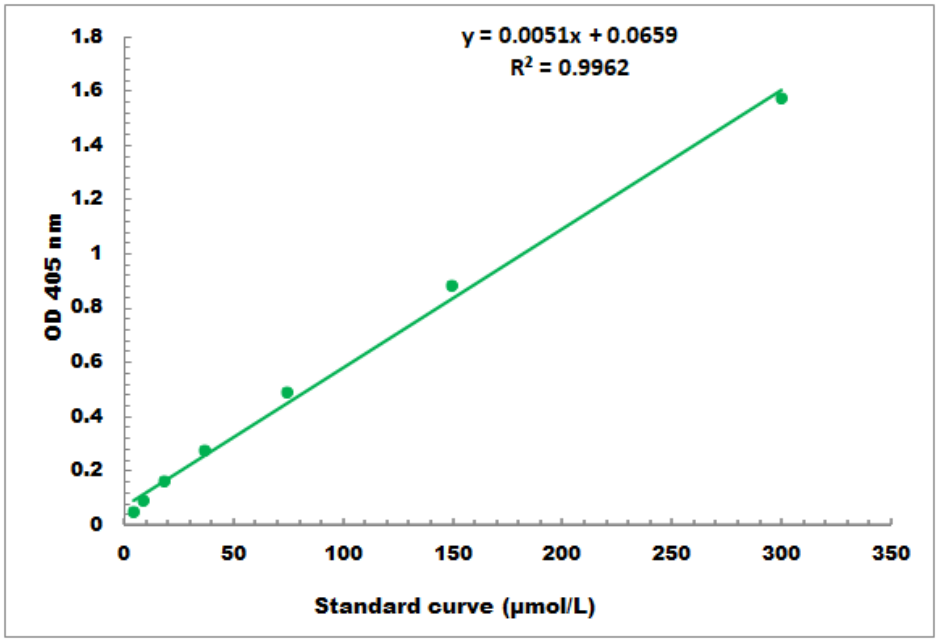
V_{Sample}: the volume of sample, 0.02 ml;

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

T: the reaction time, 30 minutes.

VII. TYPICAL DATA

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



Detection Range: 3μmol/L -300μmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES