Mixed Function Oxidase Assay Kit

ARG83404 Mixed Function Oxidase Assay Kit can be used to measure Mixed Function Oxidase in tissue extracts, cell lysate and other biological fluids

Catalog number: ARG83404

Package: 96 wells

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INTRODUCTION

Desaturation of fatty acyl-CoA in vertebrates is an example of the mixed-function oxidase reaction. In the process, saturated fatty acyl-CoA and NADPH are oxidized by molecular oxygen (O2) to produce monounsaturated fatty acyl-CoA, NADP+ and 2 molecules of water.

PRINCIPLE OF THE ASSAY

Mixed Function Oxidase Assay Kit determined Mixed Function Oxidase 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 is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Assay Buffer	4 X 30 ml	4°C
Substrate	1 vial (lyophilized)	-20°C
Reaction Buffer	10 ml	4°C
Coenzyme	1 vial (lyophilized)	-20°C
Stop Solution	10 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Substrate and Coenzyme at -20°C, other component at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

For tissue- 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.

For cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 106 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.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **Reaction Buffer** to dissolve standard; then add **30 µl** into **970 µl** of **Reaction Buffer**, to yield **300 µmol/L standard**. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Substrate:** Reconstitute the Substrate with **3 ml** of **ethanol**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Coenzyme:** Reconstitute the Substrate with **1 ml** of **Reaction Buffer**. Allow the Coenzyme keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **20 µl Sample** into Sample wells.
2. Standard wells: Add **100 µl** of **Standard Buffer** into Standard wells.
3. Add **40 µl** of **Reaction Buffer, 10 µl** of **Coenzyme, 30 µl** of **Substrate** into Sample wells.
4. Mix well. Incubate at **37°C** for **30 min**.
5. Add **100 µl** of **Stop Solution** into each wells.
6. Mix well. Read the OD at **405 nm**.

Summary of Mixed Function Oxidase Procedure

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 well. Incubate at **37°C** for **30 min**			
Stop Solution	100 µl	100 µl	100 µl
Mix well. Read the OD at **405 nm**.			

CALCULATION OF RESULTS

1. Unit Definition: One unit Mixed Function Oxidase activity is defined as the generates 1 μmol of p-nitrophenol per minute in the reaction system.

2. Calculate the average absorbance values for each set of samples and control.

3. Calculation:

A. Definition:

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

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

V_{Sample}: the volume of reaction sample, 20 μl = 0.02 ml;

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

W: the weight of sample, g;

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

T: the reaction time, 30 minutes.

B. Formula:

a). According to the protein concentration of sample

Mixed Function Oxidase activity (U/mg) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

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

b). According to the weight of sample

Mixed Function Oxidase activity (U/g) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times W) \times T]$

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

c). According to the cell or bacteria

Mixed Function Oxidase activity ($U/10^4$) =

$(OD_{Sample} - OD_{Blank}) \times (C_{Standard} \times V_{Standard}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times N) \times T]$

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