LipoxygenaseMicroplate Assay Kit

User Manual

Catalog # CAK1240

(Version 1.2B)

Detection and Quantification of LipoxygenaseActivity inTissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

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

I. INTRODUCTION

Lipoxygenases (EC 1.13.11.-) are a family of (non-heme) iron-containing enzymes most of which catalyze the dioxygenation of polyunsaturated fatty acids in lipids containing a cis,cis-1,4- pentadiene into cell signaling agents that serve diverse roles as autocrine signals that regulate the function of their parent cells, paracrine signals that regulate the function of nearby cells, and endocrine signals that regulate the function of distant cells.

Lipoxygenase Microplate Assay Kit is based on oxidation of substrate toiodine. The intensity of the product color, measured at 470 nm, is proportional to the Lipoxygenase activity in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate I	Powder x 1	4 °C
Substrate I Diluent	6 mlx 1	4 °C
Substrate II	Powder x 1	4 °C
Dye Reagent	1 ml x 1	4 °C
Reaction Buffer	7 mlx 1	4 °C
Standard (3 mmol/L)	1 mlx 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate I:add 6 ml Substrate I Diluent to dissolve before use.

Substrate II:add 3 ml distilled water to dissolve before use.

Dye Reagent: mix before use, if any precipitate, please heat at 80 °C todissolve.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 470 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 10minutes, 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 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly, or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Blank	Standard
Substrate I	60 µl	60 µl	60 µl
Sample	30 µl	--	--
Distilled water	--	30 µl	--
Standard	--	--	30 µl
Mix, incubate at 30 °C for 5 minutes.			
Reaction Buffer	70 µl	70 µl	70 µl
Substrate II	30 µl	30 µl	30 µl
Dye Reagent	10 µl	10 µl	10 µl
Mix, wait for 5 minutes, measured at 470 nm and record the absorbance.			

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 Lipoxygenaseactivity is defined as the enzymeproduces 1μmol of hydrogen peroxide per minute.

1. According to the protein concentration of sample

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

$$= 0.6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3.According to the quantity of cells or bacteria

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

$$= 0.6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

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

$C_{Standard}$: the concentration of standard, 3mmol/L = 3μmol/ml;

W: the weight of sample, g;

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

V_{Sample}: the volume of sample, 0.03 ml;

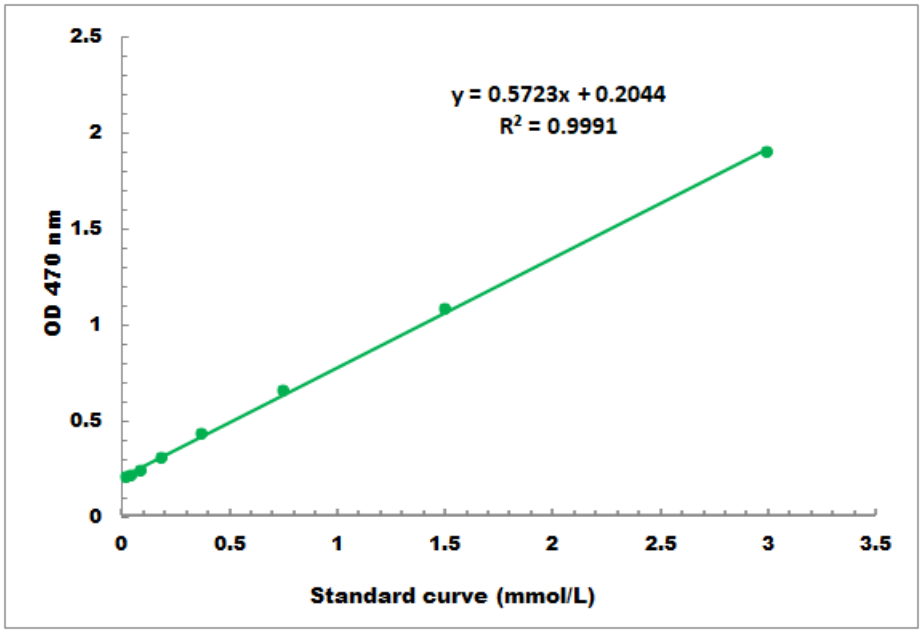
$V_{Standard}$: the volume of standard, 0.03 ml;

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: 0.03mmol/L -3mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES