



Lipoxygenase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1240

(Version 1.3B)

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

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

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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 Activity Colorimetric Microplate Assay Kit is based on oxidation of substrate to iodine. 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	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 3/1.5/0.75/0.375/0.188/0.094/0.047 mmol/L.

Substrate I: Add 6 ml Substrate I Diluent to dissolve before use. Keep in dark and store at 4 °C for 1-2 days or -20°C for 1 month after reconstitution.

Substrate II: Add 3 ml distilled water to dissolve before use. Keep in dark and store at 4 °C for 1 month or -20°C for 6 months after reconstitution.

Dye Reagent: Mix before use, if any precipitate, please heat at 80 °C to dissolve. Store at 4 °C for 1 week or -20°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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 5×10^6 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, or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

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

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of Lipoyxygenase activity is defined as the enzyme produces 1 μmol of hydrogen peroxide per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10⁴

C_{Standard}: the concentration of standard, mmol/L = μmol/mL

V_{Standard}: the volume of standard in assay procedure, mL

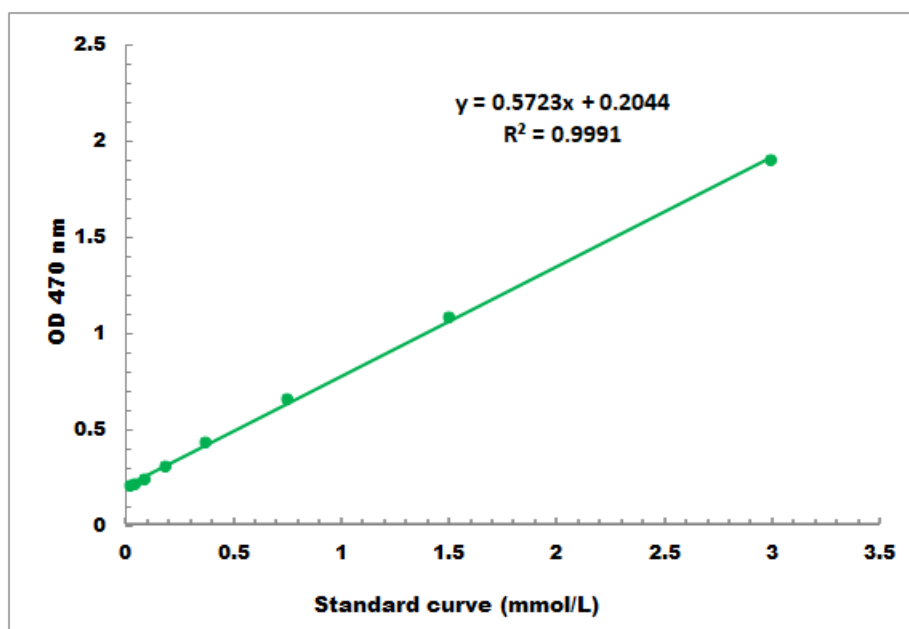
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. 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