Lipoxygenase Assay Kit

ARG83421 Lipoxygenase Assay Kit can be used to measure Lipoxygenase in tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83421

Package: 96 wells

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INTRODUCTION

Lipoxygenases (LOXs) are dioxygenases that catalyze the formation of corresponding hydroperoxides from polyunsaturated fatty acids such as linoleic acid and arachidonic acid.

PRINCIPLE OF THE ASSAY

Lipoxygenase Assay Kit determined Lipoxygenase activity in Tissue extracts, cell lysate, cell culture media and other biological fluids. Lipoxygenase Assay Kit is based on oxidation of substrate to iodine. The Lipoxygenase activity can be measured at a colorimetric readout at 470 nm

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 ml	4°C
Assay Buffer	4 X 30 ml	4°C
Reaction Buffer	7 ml	4°C
Substrate I	1 vial (lyophilized)	4°C
Substrate I Diluent	6 ml	4°C
Substrate II	1 vial (lyophilized)	4°C
Reagent Dye	1 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 470 nm
- Pipettes and pipette tips
- Deionized or distilled water
- If precipitate are observed in the Reagent Dye, warm to 80°C until the precipitate are completely dissolved.

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store all 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 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.

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.

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

REAGENT PREPARATION

- **Substrate I:** Reconstitute the Substrate with **6 ml** of **Substrate I Diluent**. Allow the Substrate I keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Substrate II:** Reconstitute the Substrate with **3 ml** of **Distilled water**. Allow the Substrate I 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. Add **60 µl Substrate I** into each tubes.
2. Add **30 µl Sample** into Sample tubes.
3. Add **30 µl Standard** into Standard tubes.
4. Mix well. Incubate at **30°C** for **5 min**.
5. Add **70 µl Reaction Buffer** into each tubes.
6. Add **30 µl Substrate II** into each tubes.
7. Add **10 µl Reagent Dye** into all wells.
8. Mix well. Incubate at **RT** for **5 min**. Read the OD at **470 nm**.

Summary of Lipoxygenase Assay Procedure

Reagent	Sample	Standard	Blank
Substrate I	60 µl	60 µl	60 µl
Sample	30 µl	-	-
Standard	-	30 µl	-
Distilled water	-	-	30 µl
Mix well. Incubate at 30°C for 30 min			
Reaction Buffer	70 µl	70 µl	70 µl
Substrate II	30 µl	30 µl	30 µl
Reagent Dye	10 µl	10 µl	10 µl
Mix well. Incubate at RT for 5 min			
Read the OD at 470 nm.			

CALCULATION OF RESULTS

1. Unit Definition: One unit Lipoxygenase activity is defined as the generates 1 µmol of hydrogen peroxide 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, 3 µmol/ml;

V_{Sample}: the volume of reaction sample, 30 µl = 0.03 ml;

$V_{Standard}$: the volume of reaction Standard, 30 µl = 0.03 ml;

V_{assay}: the volume of Assay Buffer, 1000 µl = 1 ml;

W: the weight of sample, g;

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

T: the reaction time, 5 minutes.

B. Formula:

a). According to the protein concentration of sample

Lipoxygenase activity (U/mg) =

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

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

b). According to the weight of sample

Lipoxygenase activity (U/mg) =

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

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

c). According to the cell or bacteria

Lipoxygenase activity ($U/10^4$) =

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

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