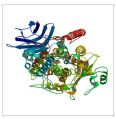

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Lipoxygenase activity determination

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1 *Works for me* dx.doi.org/10.17504/protocols.io.ba3aigie

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ABSTRACT

Lipoxygenase activity on linoleic acid was determined according to the method described by Axelrod et al. (1981). This method determines the increase in absorbance at 234 nm, resulting from the formation of a conjugated double bond system in the formed hydroperoxide.

MATERIALS

NAME	CATALOG #	VENDOR
Sodium phosphate monobasic monohydrate	S9638	Sigma Aldrich
Sodium phosphate dibasic	S3264	Sigma Aldrich
Tween 20	P1379-500ml	Sigma-aldrich
Sodium hydroxide	S8045	Sigma – Aldrich
Linoleic acid	L1012	

SAFETY WARNINGS

Wear personal protective equipment: gloves, lab coat and mask.

BEFORE STARTING

Organize your workspace

Make sure all solutions and equipment are available.

Reagent Preparation

1 ⚠

10 mM sodium linoleate stock solution

In a 150 mL Erlenmeyer add:

10 mL distilled water (previously boiled)
78 µL linoleic acid
90 µL of tween 20

Keep the solution protected from light by wrapping the Erlenmeyer in aluminum foil.

Mix the solution with the aid of a pipette, taking care not to form bubbles.

Add 0.5 M NaOH until the solution is clarified (approximately 100 µL).

Transfer the solution to a 25 mL volumetric flask protected from light. Make up the volume to 25 mL.

Divide the stock solution of sodium linoleate into 1.5 mL amber microtubes and store at -20 ° C.

2 **50.0 mM phosphate buffer, pH 6.0**

Mix sodium phosphate monobasic and dibasic solutions in the proportions indicated below, and dilute to 200 mL with deionized water.

- 6.15 mL of 0.2 M sodium phosphate, dibasic dihydrate ($Na_2HPO_4 \cdot 2H_2O$ FW = 178.05)
- 43.85 mL of 0.2M sodium phosphate, monobasic, monohydrate ($NaH_2PO_4 \cdot H_2O$ FW = 138.01)

Note: The dibasic stock sodium phosphate may be somewhat harder to dissolve; adding a little heat may help.

Complete the final volume to 200 mL with deionized water.

Adjust the final pH to 6.0.

Procedure

3 **Pipette (in microliters) the following reagents into 1.5 mL microtubes**

	Blank	Test
Phosphate Buffer	1002 µL	1000 µL
Sodium Linoleate Stock Solution	10.0 µL	10.0 µL
Enzymatic Extract (sample)	-	2.0 µL

Mix by inversion

4 Zero the spectrophotometer with Blank content at A234 nm.

5 Immediately after enzyme addition (Test), mark the time and pour the contents into a suitable cuvette.

After 30 s of reaction onset, monitor readings at A234 nm for 120 s.

Calculations

6 Calculate velocity from absorbance values obtained

$V_0 = \Delta A_{234\ nm}\ (\varepsilon\ l\ \Delta t)^{-1}$

- V_0: enzymatic activity
- $\Delta A_{234\ nm}$: absorbance variation at 234 nm
- ε: molar extinction coefficient of linoleic acid hydroperoxides at 234 nm
- l: optical path
- Δt: time (120 s)