

## Positional Analysis of Fatty Acids in Phospholipids by PLA<sub>2</sub> Treatment

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**[Abstract]** Plant phospholipids can be produced in the endoplasmic reticulum or plastids. Lipids from different sources can be distinguished by the fatty acid profile, in terms of the preferred fatty acid species esterified to the *sn*-1 or *sn*-2 position of the glycerol backbone (Ohlrogge and Browse, 1995). This protocol is used to determine the fatty acid profile in total plant phospholipids by the treatment of *sn*-2 specific phospholipase A<sub>2</sub> (PLA<sub>2</sub>).

### Materials and Reagents

1. Glass tubes [PYREX<sup>TM</sup> screw cap culture tubes with PTFE lined phenolic caps (Thermo Fisher Scientific, Fisher Scientific<sup>TM</sup>, catalog number: 14-932H)]
2. Pipette 100-1,000  $\mu$ l (Eppendorf Research 2100)
3. Axygen<sup>TM</sup> 1,000  $\mu$ l universal pipette tips (Thermo Fisher Scientific, Fisher Scientific<sup>TM</sup>, catalog number: 14-222-690)
4. Total plant lipids  
*Note: Extracted by the method described in the companion lipid extraction and profiling protocol by the same authors (Liu and Wang, 2016) and the original publication (Welti et al., 2002)*
5. Chloroform (Thermo Fisher Scientific, catalog number: C607)
6. PLA<sub>2</sub> from honey bee venom (Sigma-Aldrich, catalog number: P9279)  
*Note: Dissolved in reaction buffer to 1 unit/ $\mu$ l before use.*
7. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S7653)
8. Nitrogen gas
9. HEPES (Sigma-Aldrich, catalog number: H3375)
10. Calcium chloride (CaCl<sub>2</sub>) (Sigma-Aldrich, catalog number: C8106)
11. Magnesium chloride hexahydrate (MgCl<sub>2</sub>·6H<sub>2</sub>O) (Sigma-Aldrich, catalog number: M2670)
12. Methanol (Thermo Fisher Scientific, Fisher Scientific<sup>TM</sup>, catalog number: A456)
13. Reaction buffer (see Recipes)
14. NaCl solution (see Recipes)
15. Extraction solution (see Recipes)

## **Equipment**

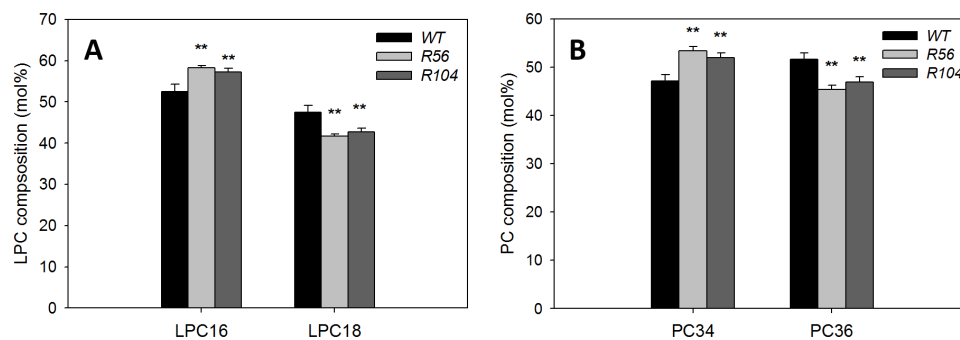
1. Nitrogen evaporator N-EVAP (Meyer Organomation, model: 111)
2. Ultrasonic processor with probe, work at 30% power (GEX 130PB) or similar equipment [e.g., ultrasonic processor, (Cole-parmer, model: EW-04714-51)]
3. Incubator (Thermo Fisher Scientific, model: Isotemp 537D) or similar equipment [e.g., microbiological incubators (Thermo Fisher Scientific, Isotemp™, catalog number: 15-103-0513)]

## **Procedure**

1. Total plant lipids were reconstituted by chloroform and the amount of lipids corresponding to 0.2 mg dry weight were aliquoted in glass tubes. Same amount of untreated lipid samples will be used as controls in step 9.
2. Lipids were dried in the nitrogen evaporator thoroughly (>30 min), mild heating (<50 °C) may be used to accelerate the process.
3. Dried lipids were hydrated in 500 µl reaction buffer at room temperature for >10 min, followed by sonication (30% power, on ice) to disperse the lipids uniformly.
4. 10 units (10 µl) of PLA<sub>2</sub> were added to the reaction, mixed by vortex. The reaction mixtures were incubated at 37 °C for 2 h to completely digest phospholipids.
5. 500 µl of 500 mM NaCl was added to the mixture to facilitate phase separation. 2 ml extraction solution was added and vortexed to terminate the reaction and extract lipids.
6. Centrifuge at ~200 x g for 10 min. Transfer the bottom (organic) phase to another glass tube.
7. Add 1 ml chloroform and repeat step 6, twice for complete extraction.
8. Dry extracted lipids under nitrogen gas, reconstitute the lipids in 50 µl chloroform.
9. Use total lipids corresponding to 0.2 mg dry weight as control, profile both digested and undigested lipids in mass spectrometry as described in the companion lipid extraction and profiling protocol by the same authors (Liu and Wang, 2016). Three or more biological repeats are recommended and Student's *t*-test is used for statistical analysis. Compare regular lipids in undigested samples and lysolipids in digested samples to determine position/composition of fatty acid in different lipid classes. For more quantitative results and more phospholipids classes besides phosphatidylcholine (PC) and phosphatidylethanolamine (PE), lipid samples can first be resolved by thin layer chromatography (TLC) (Refer to the method in Liu *et al.*, 2015) and each lipid/lysolipid spot can be quantified by transmethylation and GC analysis (Politz *et al.*, 2013).

## Representative data

Representative data from Liu *et al.*, 2015 (Figure 1). Total lipids were digested by PLA<sub>2</sub>, followed by analysis of PC and LysoPC by mass spectrometry. The increase of LPC16 in digested sample and increase of PC34 in undigested samples in RNAi lines compared with WT are similar, suggesting the increased PC34 in RNAi lines have C16 fatty acid esterified to the *sn*-1 position, which originates from the eukaryotic pathway.



**Figure 1. Analysis of fatty acyl chain position/composition in phosphatidylcholine (PC).** A. Content of LysoPC with C16 and C18 fatty acids after PLA<sub>2</sub> treatments of lipids from two *AAPT* RNAi lines and WT plants. B. Content of PC with C34 and C36 fatty acids without PLA<sub>2</sub> treatments of lipids from two *AAPT* RNAi lines and WT plants. \* and \*\* mark differences between WT and mutant at  $P < 0.05$  and at  $P < 0.01$ , respectively based on Student's *t*-test. Values are means  $\pm$  SD ( $n = 3$ ).

## Recipes

### 1. Reaction buffer

100 mM HEPES, pH 7.4

10 mM CaCl<sub>2</sub>

10 mM MgCl<sub>2</sub>

*Note: Stable in room temperature.*

### 2. NaCl solution

500 mM NaCl

*Note: Stable in room temperature.*

### 3. Extraction solution

66.7% (v/v) Chloroform

33.3% (v/v) Methanol

*Note: made before use*

## **Acknowledgments**

This protocol is adapted from the work of Williams *et al.*, 1995. Work was supported by the National Institute of Food and Agriculture Grant 2016-67013-24429 and the U.S. Department of Energy (DOE) Grant DE-AR0000202.

## **References**

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