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Benzoyl-Hexose derivatization

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Uche Medoh[1,2,3,4,5], Ali Ghoochani[1,2,3,4], Monther Abu-Remaileh[1,2,3,4]

[1]Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA;
[2]Department of Genetics, Stanford University, Stanford, CA 94305, USA;
[3]The Institute for Chemistry, Engineering and Medicine for Human Health (Sarafan ChEM-H), Stanford University, Stanford, CA 94305, USA;
[4]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA;
[5]Arc Institute, Palo Alto, CA 94304, USA

asap

Ali Ghoochani
Stanford University

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We use this protocol and it's working

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Abstract

Hexose derivatization is a crucial analytical procedure used to enhance the detection and quantification of hexose sugars in various samples by liquid chromatography-mass spectrometry (LC-MS).

Materials

- Ethyl acetate
- Water
- 1 M Potassium phosphate dibasic (K_2HPO_4)
- 8 M Sodium hydroxide (NaOH)
- Benzoyl chloride
- 1.4 M Phosphoric acid (H_3PO_4)
- 1 M Sodium hydroxide (NaOH)
- Acetonitrile
- 100 mM Ammonium formate (NH_4HCO_2)
- Vortex
- Centrifuge (capable of 16,000 g)
- Speed vacuum
- Mass spectrometer (for m/z detection)

1 Add 200 µL of a **1:1 ethyl acetate:water** to cells or beads after organelle IP such as LysoIP

1.1 Vortex the mixture for 00:05:00 5m

1.2 Centrifuge at 16000 x g, 00:05:00 5m

2 Carefully remove the **upper layer** (ethyl acetate) and discard.

3 Transfer **the bottom (water) layer** to a new tube.

4 **Benzoyl Derivatization**

4.1 Add 20 µL of **1 M potassium phosphate dibasic (K_2HPO_4)**

4.2 Add 20 µL of **8 M NaOH**

4.3 Add 10 µL of **benzoyl chloride**

4.4 Vortex the mixture for 00:05:00 and then briefly spin down to collect the liquid. 5m

5 Add 10 µL of **1.4 M phosphoric acid (H_3PO_4)**

6 Add 500 µL of **ethyl acetate**

7 Vortex for 00:03:00 and Centrifuge at 16000 x g, 00:05:00 8m

8 Transfer 400 µL of the **upper layer** (ethyl acetate) to a new tube.

9 Add **100 µl of 1 M NaOH** to the ethyl acetate extract.

10 Vortex for 00:02:00 and then centrifuge at 16000 x g, 00:05:00 7m

11 Transfer 100 µL of the **upper layer** (ethyl acetate) to a new tube.

12 **Repeat the process:**
Add 100 µL of **1 M NaOH** to the remaining ethyl acetate solution.

13 Vortex for 00:02:00 Centrifuge at 16000 x g, 00:05:00 7m

14 Transfer 100 µL of **upper layer** to the same tube as the previous 100 µL, for a total of 200 µL of ethyl acetate.

15 **Drying and Reconstitution**

15.1 Dry the 200 µl of ethyl acetate in a **speed vacuum**.

15.2 Reconstitute the dried sample in 50 µL of a **9:1 acetonitrile:water containing 100 mM ammonium formate (NH_4HCO_2)**.

16 **Mass Spectrometry Monitoring**

- Analyze the sample for **parent 718 m/z [M+NH_4]+** and the following product ions:
- **231**
- **105**
- **579.1**