SPLASH® Lipidomix Quantitative Mass Spec Internal Standard Extraction Protocols

*** This is a general method for using SPLASH® products; optimization may be required based on the mixture and matrix you are using.***

Extraction Protocol for Plasma

1. Use 13x100 mm new glass screw capped tubes. Do not use washed tubes as you may extract detergent residue.
2. Add 90 µL water to 10 µL plasma, vortex then let sit on ice for 10 minutes.
3. Add 2.0 mL methanol.
4. Add 0.9 mL dichloromethane.
5. Vortex
6. A single phase should appear, If there are two distinct phases, add 50 µL methanol and vortex. If solution is still not a single phase, repeat addition of 50 µL methanol and vortex.
7. Add 10 µL SPLASH® Internal Standard, vortex, and let mixture sit for 30 minutes at room temperature.
8. Add 1 mL water.
9. Add 0.9 mL dichloromethane.
10. Invert tube 10 times. DO NOT CORTEX or you will form an emulsion.
11. Centrifuge at 1200 rpm for 10 minutes.
12. Collect lower layer and put into a new glass tube.
13. Add 2 mL dichloromethane to remains in extraction tube.
14. Mix, centrifuge, collect lower layer. Add to first extract.
15. Evaporate solvent under a stream of nitrogen.
16. Re-suspend lipids in injection solvent.

Extraction Protocol for Tissues

1. Weigh tissue to be extracted. 50-100 mg is sufficient. Calculate water content. Expected values are as follows:
   - Adipose: 18%
   - Brain: 60%
   - Bone: 44%
   - Heart, kidney, liver, lung, intestines, spleen, and stomach: 65%
   - Testes: 18%
2. Add water to tissue so that the total volume is 1 mL. Example: 100 mg brain tissue corresponds to 60 µL water. Add 940 µL water.
3. Disperse tissue:
   a. Grind tissue frozen in liquid nitrogen using cold mortar and pestle.
   b. Blend using a homogenizer.
4. Sonicate for 30 seconds with 5 second bursts and 20 second rest times. Perform sonication steps on ice.
5. Add 2.0 mL methanol.
6. Add 0.9 mL dichloromethane.
7. Vortex
8. **Repeat steps 6-16 from Extraction Protocol for Plasma**

Extraction Protocol for Cells

1. Use 13x100 mm new glass screw capped tubes. Do not use washed tubes as you may extract detergent residue.
2. Collect cells:
   a. Wash cells with non-buffered saline to remove cell culture medium.
   b. For cells in suspension: centrifuge, discard saline, add 1 mL water. Vortex and transfer to glass tube for extraction. Rest on ice for 10 minutes. Ensure final volume is 1 mL and adjust if necessary.
   c. For adhered cells: wash cells with non-buffered saline. Add 1 mL water to lyse cells and scrap. Collect cell lysate and transfer to glass tube for extraction. Rest on ice for 10 minutes. Ensure final volume is 1 mL and adjust if necessary.
3. Add 2.0 mL methanol.
4. Add 0.9 mL dichloromethane.
5. Vortex.
6. **Repeat steps 6-16 from Extraction Protocol for Plasma.**

Have an additional question?

Please email us at lipidomics@avantilipids.com if you have any additional questions about a lipidomix internal standard mnixture.

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