EquiSPLASH™ LIPIDOMIX® Quantitative Mass Spec Internal Standard Avanti Product Number 330731-1EA

Technical Data Sheet / Frequently Asked Questions

What is included in EquiSPLASH™ Standard?

Avanti Product Number 330731-1EA includes a single sealed ampule of Avanti's EquiSPLASH™ LIPIDOMIX® Quantitative Mass Spec Internal Standard (Product Number 330731). Each sealed ampule of EquiSPLASH™ contains 1mL of methanol solution with 13 deuterated lipid internal standards at a target concentration of 100 µg/mL each. The concentrations are verified and based on the isotopic purity of each individual compound.

EquiSPLASH™ was designed to complement a wide variety of lipidomics platforms and sample types by offering one concentration for all lipid classes. This will allow you to individualize the application of the standard to your methods and instrumentation. Using one internal standard per lipid class will allow you to correct for extraction efficiency and ionization efficiency between lipids classes. This ready-to-use standard mixture will save your lab both time and money when compared to purchasing 13 individual standards and preparing your own standard mixture.

How do I handle EquiSPLASH™ properly?

Your EquiSPLASH™ standard should be stored in a -10°C to -25°C freezer until ready for use. It is designed to be a one-time use ampule, and we do not recommend storing for long periods of time after opening.

Always make sure to warm bath sonicate the unopened ampule for approximately 2 minutes prior to opening the ampule. Lipids in solution may precipitate during shipping and storage conditions, and it may not be visible with a solution at extremely low concentrations such as this.

Direct transfer from ampule to experimental sample prep glass vial for immediate use is suggested. General handling guidelines for lipids should be followed. As outlined on our website.

How do I prep my biological samples using EquiSPLASH™ Internal Standards?

Please see our recommended sample extraction procedures on the reverse side of this brochure.

EquiSPLASH™ LIPIDOMIX® Quantitative Mass Spec Internal Standard / Avanti Product 330731-1EA

Mixture Component	Chemical Formula	Target Conc. µg/mL	Target Conc. mM	Exact Mass	M-H	M+H	$M+NH_4$	M+AcO
15:0-18:1(d7) PC	$C_{41}H_{73}D_7NO_8P$	100	133	752.6061	-	753.6134	-	811.6199
18:1(d7) Lyso PC	$C_{26}H_{45}D_7NO_7P$	100	189	528.3921	-	529.3994	-	587.4059
15:0-18:1(d7) PE	$C_{38}H_{67}D_7NO_8P$	100	141	710.5591	709.5519	711.5664	--	-
18:1(d7) Lyso PE	$C_{23}H_{39}D_7NO_7P$	100	205	486.3451	485.3379	487.3524		-
15:0-18:1(d7) PG	$C_{39}H_{68}D_7O_{10}P$	100	131	741.5537	740.5464	-	759.5875	-
15:0-18:1(d7) PI	$C_{42}H_{72}D_7O_{13}P$	100	118	829.5698	828.5625	-	847.6036	-
15:0-18:1(d7) PS	$C_{39}H_{67}D_7NO_{10}P$	100	129	754.5490	753.5417	755.5562	-	-
15:0-18:1(d7)-15:0 TAG	$C_{51}H_{89}D_7O_6$	100	123	811.7646	-	-	829.7985	-
15:0-18:1(d7) DAG	$C_{36}H_{61}D_7O_5$	100	170	587.5506	-	-	605.5844	-
18:1(d7) MAG	$C_{21}H_{33}D_7O_4$	100	275	363.3366	-	364.3429	381.3704	422.3504
18:1(d7) Chol Ester	$C_{45}H_{71}D_7O_2$	100	152	657.6441	-	-	675.6779	-
d18:1-18:1(d9) SM	$C_{41}H_{72}D_9N_2O_6P$	100	135	737.6397	-	738.6470	-	796.6536
C15 Ceramide-d7	$C_{33}H_{58}D_7NO_3$	100	188	530.5404	529.5331	531.5477	-	589.5542

Extraction Protocol for Plasma

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1. Use 13 x 100 mm new glass screw capped tubes. Do not use washed tubes as you may extract detergent residue.
2. Add 990 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 singe 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 EquiSPLASH™ 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 VORTEX 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, and 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 Plasma

1. Use 13 x 100 mm new glass screw capped tubes. Do not use washed tubes as you may extract detergent residue.
2. Collect cells:
   I. Wash cells with non-buffered saline to remove cell culture medium.
   II. For cells in suspension: centrifuge, discard saline, and 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.
   III. 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.

Extraction Protocol for Solid Tissue

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 total volume is 1 mL. Example: 100 mg of brain tissue corresponds to 60 µL water. Add 940 µL water.
3. Disperse tissue.
   I. Grind tissue frozen in liquid nitrogen using cold mortar and pestle..
   II. Blend using a homogenizer.
4. Sonicate for 30 seconds with 5 seconds bursts and 20 second rest time. Perform sonication steps on ice.
5. Add 2.0 mL of methanol.
6. Add 0.9 mL dichloromethane..
7. Vortex.
8. Repeat steps 6-16 from Extraction Protocol for Plasma.

Who do I contact if I have additional questions?

Please e-mail us at lipidomics@avantilipids.com if you have any additional questions about this standard or other Avanti products and services.

How do I order more EquiSPLASH™?

To order more EquiSPLASH™ please visit the Avanti website and search for product number 330731. Customers in the United States can order directly from Avanti, and customers outside the United States will be directed to our worldwide distribution partner for country specific ordering information and pricing.

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