Protocol MSU_MSMC_005 version 1.1
Preparation of internal standard cocktail solutions for analysis of animal tissue metabolites

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Reagents

Compound name	Source	MSMC Standard Bar Code
Palmitic acid-d_{31}	Sigma-Aldrich #366897	IS_0003
[$^{13}C_6$]D-glucose	Sigma-Aldrich #389374	
L-Phenylalanine-d_7	Toronto Research Chemicals #P319416	IS_0005
Pyruvic acid-$^{13}C_3$ sodium salt	Toronto Research Chemicals #P998904	IS_0006
(-)-Inosine-1',2',3',4',5'-$^{13}C_5$	Toronto Research Chemicals #I661003	IS_0004

Materials
15-mL polypropylene centrifuge tubes
2-mL polypropylene microcentrifuge tubes
Pyrex screw cap culture tubes with PTFE-lined phenolic caps, 13 x 100 mm (Fisher Scientific #14-933A)
Glass culture tubes with PTFE-lined screw caps, 10 x 75 mm (BMB Stores #1496215)
Glass media bottle (25 or 125 mL)
Graduated cylinders

Solvents
HPLC grade acetonitrile
MilliQ water (> 18 MΩ)
HPLC grade chloroform
HPLC grade methanol

Prepare at least 100 mL of a solution of 50% acetonitrile (v/v) in MilliQ water.
Prepare at least 10 mL of a solution of 2:1 chloroform/methanol in a glass bottle with PTFE-lined cap

Prepare stock solutions as follows:

Stock 1	Dissolve 5 mg of palmitic acid-d_{31} in 5000 µL (preferably measured using a positive-displacement pipetter) of 2:1 chloroform:methanol in a 13 x 100 mm glass culture tube (that has a PTFE-lined cap). Cap the tube and vortex for 30 seconds. Label this as Stock #1 (1.0 mg/mL palmitic acid-d_{31}). Wait at least 15

	minutes before withdrawing aliquots. Store at -20˚C. Allow to thaw to room temperature for 1 hour before withdrawing liquid.
Stock 2	Dissolve 4 mg of [$^{13}C_6$]D-glucose in 1000 µL of 50% acetonitrile in water in a 2-mL polypropylene microcentrifuge tube; Vortex until all of the solid has dissolved. Label this as Stock #2 (4 mg/mL [$^{13}C_6$]D-glucose)
Stock 3	Dissolve 5 mg of phenylalanine-d_7 in 1000 µL of 50% acetonitrile in water in a 2-mL polypropylene microcentrifuge tube; Vortex until all of the solid has dissolved. Label this as Stock #3 (5 mg/mL phenylalanine-d_7).
Stock 4	Dissolve 5 mg of [$^{13}C_3$]pyruvic acid sodium salt in 1000 µL of 50% acetonitrile in water in a 2-mL polypropylene microcentrifuge tube; Vortex until all of the solid has dissolved. Label this as Stock #4 (5 mg/mL [$^{13}C_3$]pyruvic acid sodium salt)
Stock 5	Dissolve 1 mg of inosine-1',2',3',4',5'-$^{13}C_5$ in 1000 µL of 50% acetonitrile in water in a 2-mL polypropylene microcentrifuge tube; Vortex until all of the solid has dissolved. Label this as Stock #5 (1 mg/mL inosine-1',2',3',4',5'-$^{13}C_5$)

Prepare a mixed stock solution of the polar water-soluble internal standards by mixing 100. µL each of Stock 2, Stock 3, Stock 4, and Stock 5 in a 2-mL polypropylene microcentrifuge tube. Add an additional 600 µL of 50% acetonitrile/50% water, and vortex to mix. Label the tube as "Mixed polar internal standard cocktail". Concentrations will be:

Polar compounds

[$^{13}C_6$]D-glucose	400 µg/mL	(10 µg in 25 µL)
Phenylalanine-d_7	500 µg/mL	(12.5 µg in 25 µL)
[$^{13}C_3$]pyruvic acid sodium salt	500 µg/mL	(12.5 µg in 25 µL)
inosine-1',2',3',4',5'-$^{13}C_5$	100 µg/mL	(2.5 µg in 25 µL)

Mix 500 µL of the palmitic acid-d_{31} stock solution with 500 µL of 2:1 chloroform/methanol in an amber glass autosampler vial with PTFE-lined cap.

Nonpolar compounds

Palmitic acid-d_{31}	500 µg/mL	(12.5 µg in 25 µL)