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ASAP Collaborative Rese…

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We use this protocol and it's working

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Abstract

This protocol describes steps for targeting metabolites with [U-13C]glucose in mice for metabolomics analysis.

Tail vein infusion and tissue harvest

1 **Materials**

- Surgical tools – scissors, tweezers, forceps, spatula, razor
- Heating pad (K&H Thermo-Peep Heated Pad)
- Tail illuminator (Braintree Scientific: MTI STD)
- Glue (Loctite 1699233)
- 30g needles (BD: 305106)
- PE tubing (BD: PE#10)
- Syringe pump (WPI: SP220I)
- 1mL syringes (BD: 309628)
- Vapor splitter (VetEquip: CX-IM 5 place manifold)
- Dry ice
- Sterile 1X PBS or saline
- [U-13C]glucose solutions (Cambridge Isotope Laboratories: CLM-1396-1)
- Glucometer (Zoetis: AlphaTrak 2)
- Luer adapter

2 **Day Prior to Procedure**

2.1 Weigh mice.

2.2 Fast mice overnight (14-16 hours).

3 **Day of Procedure**

3.1 Make [U-13C]glucose solutions with PBS/saline.

- Bolus: 200 µL of 0.4 mg/g.
- Infusion: 75 µL of 0.72 mg/g.

3.2 Anesthetize mice in an induction chamber with 2% isoflurane with flowrate set to 1.2 L/min.

3.3 Move a mouse to the tail illuminator, cannulate the tail vein and glue needle in place.

3.4 Test that the catheter works by infusing 0.1 mL of saline – the saline should flow up the tail vein if it was cannulated correctly.

3.5 Gently move mouse and catheter to the isoflurane-splitter.

3.6 Repeat steps 3-4 to fill up the remaining spaces on the isoflurane-splitter (up to 5 mice).

3.7 Prick the paw of each mouse with a needle (18G) to measure the pre-infusion blood glucose level.

3.8 Inject the bolus glucose solution into each mouse and transfer the luer adapter to the infusion syringes.

3.9 Start infusion at a flow rate of 150 µL/h for 75 µL (30 min).

3.10 After infusion is done, euthanize mice by cervical dislocation.

3.11 Quickly dissect out the brain and flash freeze in isopentane cooled to -80C or liquid nitrogen.

3.12 Store samples in -80°C until further processing.

Metabolite Extraction

4 Protocol adapted from UCLA Metabolomics Center.

Materials

- Aluminum foil
- Metal Pad
- Dry ice
- Hammer
- Microcentrifuge tubes
- 80% methanol
- Vortex mixer
- Ice

- Centrifuge
- Screw-cap glass vials

5 Wrap frozen brains in pre-cooled aluminum foil and place on a metal pad on ground dry ice.

6 Pulverize tissue with a hammer.

7 Transfer the ground tissue into a pre-cooled microcentrifuge tube on dry ice.

8 Add 1mL 80% MeOH at -80°C and vortex vigorously for 30 s.

9 Incubate samples at -80°C for an hour.

10 Allow samples to warm up a bit on ice.

11 Vortex samples again for 30 s.

12 Centrifuge at top speed (16,000 g) for 15 min at 4°C.

13 Transfer the supernatant to a screw-cap glass vial and store at -80°C until shipping to UCLA Metabolomics Core.

Metabolomics Analysis

14 Protocol from UCLA Metabolomics Center.

Materials

- Q Exactive mass spectrometer (Thermo Scientific)
- Vanquish Flex UHPLC (Thermo Scientific)
- Ion Chromatography System ICS-5000+
- Luna NH2 3um 100A (150 × 2.0 mm) column (Phenomenex)
- LC-MS grade acetonitrile (ACN)
- LC-MS grade water

- 5 M ammonium acetate
- ammonium hydroxide

15 Dilute the MeOH extract with 50% ACN to 50 mg tissue/ml concentration (1:5-1:10).

16 Spin 10 min at full speed at 4C to clarify the samples.

17 Transfer the supernatant to glass vials with glass insert.

18 Combine 10 ul of each sample to make a pool sample used to condition the column prior loading the individual samples.

19 Place samples in autosampler 4C.

20 Load 5 ul onto a Luna NH2 column using a Vanquish Flex.

21 Separate the metabolites at a flow rate of 200 µl/min with mobile phases A (5 mM NH4AcO pH 9.9) and B (100% ACN) using a linear gradient from 15% A to 95% A over 18 min followed by a 7 min isocratic wash at 95% A.

22 Re-equilibrate the column to 15% A for 15 min prior to loading the next sample.

23 Acquire data with a Q Exactive mass spectrometer using polarity switching in full scan mode using a range of 70-975 m/z and 70.000 resolution.

24 Convert the RAW files to mzXML format using the msConverter (part of open source ProteoWizard).

25 Extract metabolite intensities with Maven (v 8.1.27.11) using a targeted list of polar metabolites of central carbon metabolism with expected retention time and accurate mass measurements (< 5 ppm) for identification.

26 Perform data analysis using R scripts functions (or other tools). Correct isotopologue data for the natural abundance of C13 using AccuCor.