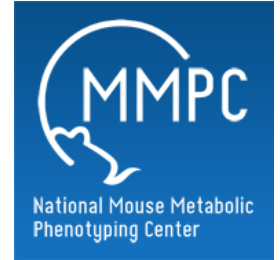


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🌐 U Mass - Organ-specific glucose uptake

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

Glucose uptake in individual organs can be measured using a bolus injection of 2-deoxy-D-[1-¹⁴C] glucose, a non-metabolizable glucose analog, and by determining labeled metabolite levels in select tissues. Insulin resistance is characterized by reduced glucose metabolism and develops in obese mice.

Materials

MATERIALS

⊗ Poly-prep columns prefilled with AG 1-X8 resin **Bio-Rad Laboratories Catalog #731-6211**

⊗ 0.2 M formic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F0507**

⊗ 0.5 M ammonium acetate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1542**

Reagent Preparation:

Reagent 1: 0.2 M formic acid/0.5 M ammonium acetate

Reagents and Materials: formic acid, ammonium acetate, deionized water

Procedure:

1. Prepare 900 ml of dH₂O, and add 7.69 ml of formic acid.
2. Add 38.84 g of ammonium acetate, and adjust pH to 4.9±0.05 using dH₂O.
3. Add dH₂O to make a final solution volume of 1,000 ml.

Note:

Bio-Rad Laboratories [RRID:SCR_008426](#)

Sigma-Aldrich [RRID:SCR_008988](#)



- 1 Survival surgery is performed to establish a chronic indwelling catheter at 5~6 days prior to experiment for intravenous infusion. (refer to M1023: Surgery-jugular vein cannulation)
- 2 Mice are fasted overnight (~15 hours) or for 5 hours prior to the start of experiment.
- 3 Place a mouse in a rat-size restrainer with its tail tape-tethered at one end.
- 4 Administer an intravenous bolus injection of 10 μCi of 2-deoxy-D-[1- ^{14}C] glucose (2-[^{14}C]DG) in awake mice. Alternatively, intraperitoneal injection of 10 μCi of 2-[^{14}C]DG may be used in awake mice.
- 5 After 30 min, rapidly freeze-clamp the tissues in liquid N_2 , and store tissue samples in -80°C freezer for biochemical assay.
- 6 Biochemical assay is conducted using frozen tissue samples (e.g., skeletal muscle, adipose tissue, heart) to measure tissue levels of 2-[^{14}C]DG-6-phosphate.
 - a) Prepare a heat block set to $\sim 100^\circ\text{C}$.
 - b) Prepare anion-exchange columns by washing with 5 ml of dH_2O .
 - c) Homogenize 50–100 mg of frozen tissue samples by adding ten times the volume of dH_2O (50 mg of tissue in 500 μl of dH_2O) in glass tubes using a tissue homogenizer.
 - d) Following homogenization, place the glass tubes in the heat block for 10 min, vortex for 2 sec, and then cool to room temperature.
 - e) Transfer the homogenized samples to microcentrifuge tubes using transfer pipettes and centrifuge at $16,000 \times g$ for 5 min.
 - f) Add 33 μl of homogenate (supernatant) to 467 μl dH_2O in a scintillation vial labeled "total" sample.
 - g) Add 5 ml of scintillation cocktail, vortex, and count the samples for ^{14}C using a liquid scintillation counter (total ^{14}C samples).
 - h) Transfer 333 μl of homogenate (supernatant) to the anionexchange columns for the separation of 2-[^{14}C]DG-6-P from 2-[^{14}C] DG.



i) Wash the columns with 2 ml of dH₂O three times and collect the samples into a scintillation vial labeled "wash" sample.

j) Vortex the "wash" samples, and transfer 500 µl of "wash" samples to another set of scintillation vials to be counted for ¹⁴C using a liquid scintillation counter (wash samples containing 2-[¹⁴C] DG).

k) Elute the columns with 2 ml of 0.2 M formic acid/0.5 M ammonium acetate three times, and collect the samples into a scintillation vial labeled "eluate" sample.

l) Vortex the "eluate" samples, and transfer 500 µl of "eluate" samples to another set of scintillation vials to be counted for ¹⁴C using a liquid scintillation counter (eluate samples containing 2-[¹⁴C] DG-6-P).

7 The rate of glucose uptake in individual organs is determined using 2-[¹⁴C] DG. 2-[¹⁴C] DG is taken up by cells, phosphorylated by glucokinase to become 2-[¹⁴C] DG-6-P, and not further metabolized. Thus, organ-specific accumulation and level of 2-[¹⁴C] DG-6-P following a bolus injection of 2-[¹⁴C] DG reflect glucose uptake in individual organs.