^{14}C-2-Deoxylglucose Uptake in Muscle and Adipose Tissue

Version: 1
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Summary:

To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-D-[1-14C]glucose will be administered as a bolus (10 uCi) at 75 min after the start of clamp experiments

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Poly-Prep® Columns	BIO-RAD	731-6211
7mL Borosilicate Glass Scintillation Vial	Fisher Scientific	03-337-26
Glass Culture Tubes 12X75mm		
Formic Acid	Sigma-Aldrich	
Ammonium Acetate	Sigma-Aldrich	
Ultima Gold	Perkin Elmer	6013329

Protocol:

1. Prepare water bath and adjust T° to 100°C.
2. Prepare frozen tissue, liquid nitrogen, homogenizer, glass tubes, printout sheet, dH_2O (2 beakers) for washing and samples dilution.
3. Add X10 volume of dH_2O to 60-110 mg tissue (BAT: whole piece) and homogenize. Wash the blender by blending water between samples and dry with paper towel.
4. Place tubes in water bath for at least 10 minutes after **capping them with foil** and then vortex for 1-2 seconds, then cool to room temperature. Remove foil and replace with parafilm. Rinse and dry tubes in hot sink.
5. Centrifuge 30 minutes at 3000 rpm.
6. Prepare the anion-exchange columns (open both extremities) and wash columns with 5 ml of dH_2O (special pipette, rinse also the borders of the columns when pouring water).
7. Add 460 µl of dH_2O to small scintillation vials and then 40 µl of supernatant, make a total of 500 µl and vortex. Add 5 ml of Ultima Gold scintillation cocktail, vortex, label as T (Total), then count on beta counter.
8. Transfer 400 µl of supernatant (if it is possible, or 250 µl and write down) to columns. Wash with 2 ml of dH_2O three times, collecting the water coming down the columns in small scintillation vials (keep them), vortex, then transfer 500 µl of samples to other small scintillation vials (label as W for Washing), add 5 ml of Ultima Gold cocktail, vortex, then count on beta counter.
9. Elute samples with 2 ml of 0.2 M FA/0.5 M AA three times and collect in small scintillation vials (keep them), vortex, then transfer 500 µl to other small scintillation vials (label as E for Eluate), add 5 ml of Ultima Gold cocktail, vortex, then count on beta counter.

Reagent Preparation:

Reagent: **0.2 M FA/0.5 M AA:**

1. 900 ml dH_2O + 7.67 ml formic acid.
2. Add 38.54 g ammonium acetate and adjust pH to 4.9 using dH_2O.
3. Add dH_2O to bring final volume to 1000 ml.