**Protocol for lipid and urea extraction of animal tissue for isotope analysis** #

Blood Plasma

1. Freeze (at -20C) plasma samples immediately after separation from other blood components (plasma top layer; leucocytes & erythrocyctes bottom layers)
2. Transfer 500 µL of sample to a 7-mL test tube and freeze-dry over night
3. Add 5 mL of petroleum ether to each test tube and cap tightly
4. Sonicate with heat "on" samples under a fume hood for 15 minutes
5. Decant the used solution
6. Repeat steps 3-5 for a second and third rinse
7. Add 5mL of deionized to each test tube and cap tightly
8. Sonicate with heat "on" samples under a fume hood for 15 minutes
9. Decant the used water
10. Repeat steps 7-9 two more times for a second and third water rinse
11. Freeze-dry samples over night to remove any remaining water
12. Homogenize the extracted tissue within the test tube and weigh ~1.0 mg of the sample into packing tin
13. Combust at 1100C and deliver gases via continuous-flow for analysis of del13C, del15N, percentage carbon and percentage nitrogen using and isotope ratio mass spectrometer (IRMS).
14. Calculate atomic C:N ratio from using the measured mass of each element:
    a. C:N = (mass of C)/(mass of N)
    b. If C:N is greater than 3.0, it likely still contains lipids and another aliquot of sample should be extracted using additional petroleum ether rinses
    c. Report mean C:N and standard errors for tissues subject to SIA

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Whole Blood

1. Freeze (at -20C) samples immediately after separation from other blood components (plasma top layer; leucocytes & erythrocyctes bottom layers)
2. Transfer 250 µL of sample to a 7-mL test tube and freeze-dry over night
3. Add 5 mL of petroleum ether to each test tube and cap tightly
4. Sonicate with heat "on" samples under a fume hood for 15 minutes
5. Decant the used solution
6. Repeat steps 3-5 for a second and third rinse
7. Add 5mL of deionized to each test tube and cap tightly
8. Sonicate with heat "on" samples under a fume hood for 15 minutes
9. Decant the used water
10. Repeat steps 7-9 two more times for a second and third water rinse
11. Freeze-dry samples over night to remove any remaining water
12. Homogenize the extracted tissue within the test tube and weigh ~1.0 mg of the sample into packing tin
13. Combust at 1100C and deliver gases via continuous-flow for analysis of del13C, del15N, percentage carbon and percentage nitrogen using and isotope ratio mass spectrometer (IRMS).
14. Calculate atomic C:N ratio from using the measured mass of each element:
    a. C:N = (mass of C)/(mass of N)
    b. If C:N is greater than 3.0, it likely still contains lipids and another aliquot of sample should be extracted using additional petroleum ether rinses
    c. Report mean C:N and standard errors for tissues subject to SIA

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Muscle/Skin Tissue

1. Freeze (at -20C) samples immediately after collection
2. Freeze-dry whole sample in original containers for ~48 hrs
3. Grind whole sample with ball mill for 3 min
4. Transfer >5 mg (dry weight) of ground sample into 15 mL test tubes with PE caps
5. Add 5 mL of petroleum ether to each test tube and cap tightly
6. Sonicate with heat "on" samples under a fume hood for 15 minutes
7. Decant the used solution
8. Repeat steps 3-5 for a second and third rinse
9. Add 5mL of deionized to each test tube and cap tightly
10. Sonicate with heat "on" samples under a fume hood for 15 minutes
11. Centrifuge all tubes for 2min
12. Decant the used water
13. Repeat steps 7-9 two more times for a second and third water rinse
14. Freeze-dry samples over night to remove any remaining water
15. Homogenize the extracted tissue within the test tube and weigh ~1.0 mg of the sample into packing tin
16. Combust at 1100C and deliver gases via continuous-flow for analysis of del13C, del15N, percentage carbon and percentage nitrogen using and isotope ratio mass spectrometer (IRMS).
17. Calculate atomic C:N ratio from using the measured mass of each element:
    a. C:N = (mass of C)/(mass of N)
    b. If C:N is greater than 3.0, it likely still contains lipids and another aliquot of sample should be extracted using additional petroleum ether rinses
    c. Report mean C:N and standard errors for tissues subject to SIA

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