ABGENT CUSTOM SERVICES:

Tissue Lysates Preparation Protocol

Step-by-step procedure (Always keep tissue on ice at all times during preparation):

1. Remove tissues, and weigh 1.5 g of each tissue.
2. Chop the tissue into small pieces, wash twice with ice-cold PBS.
3. Transfer chopped tissue into grinder, and add 5 ml RIPA buffer, homogenize 20 times.
4. Transfer homogenized solution into 1.5 ml microcentrifuge tube. Spin at 14000 rpm for 10 min at 4°C.
5. Carefully remove the lipid on the surface of the supernatant. Save supernatant as whole tissue lysate, and discard the pellet.
6. Determine the protein concentration by Bradford assay.
7. Adjust concentration to 2.5 mg/ml with Lysis buffer. Aliquot 100 ul per vial, and store at -80°C.
8. For western blotting, add 100 ul 2 × Sample buffer, boil for 5 minutes, and store at -20°C.

Reagents

RIPA buffer:
50 mM Tris-Cl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton ×100, 1% Na deoxycholate, 0.1% SDS, 1 mM PMSF, 1 ug/mL aprotinin, 1 ug/mL leupeptin
Lysis buffer:
0.15 M NaCl, 5 mM EDTA(pH 8.0), 1% Triton ×100, 10 mM Tris-Cl (pH 7.4). Just before use, add 5 mM DTT, 0.1 mM PMSF in isopropanol, 5 mM ε-aminocaproic acid
PBS (pH7.4):
10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 50 mM NaCl, 2.7 mM KCl
2×Sample buffer:
130 mM Tris-Cl (pH8.0), 20% (v/v) glycerol, 4.6% (w/v) SDS, 0.02% bromophenol blue, 2% DTT

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