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primary antibody company

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Cell Lysates Preparation Protocol

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

1. Collect confluent cells (from T25 flask) by trypsinization and spin.
2. Lyse the pellet with 100 ul Lysis buffer on ice for 10 min (use 20 ul Lysis buffer/500,000 cells).
3. Spin at 14,000 rpm in a microcentrifuge tube for 10 min at 4°C.
4. Transfer the supernatant to a new tube and discard the pellet.
5. Determine the protein concentration by Bradford assay.
6. Mix 1 volume of lysate (0.5 mg protein/membrane) with 1 volume of 2 × Sample buffer.
7. Boil for 5 min and cool at room temperature (RT) for 5 min.
8. Flash spin to bring down condensation prior to loading gel.

Reagents

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 ¦Å-aminocaproic acid
2 × Sample buffer:
130 mM Tris-Cl (pH8.0), 20% (v/v) glycerol, 4.6% (w/v) SDS, 0.02% bromophenol blue, 2% DTT
PBS (pH7.4):
10 mM Na_2HPO_4, 1.8mM KH_2PO_4, 50 mM NaCl, 2.7 mM KCl

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