

Preparation of Brain Lysate

1. Remove the brain from the rat. Place it in a 50ml tube and freeze it in liquid N₂. Keep it at -70C until further use.
2. Prepare lysis buffer:
 - 50 mM Tris ph 7.5
 - 5 mM EDTA
 - 1% Triton X-100
 - Complete mini (proteases inhibitors of Roche)
3. Add 5 volumes of ice-cold lysis buffer to the frozen tissue (about 7-10 ml/brain). Homogenize the tissue on ice with a Polytron until a "smooth" mixture is achieved.
4. Centrifuge in an ultracentrifuge at 150,000xg at 4°C during 30-60'.
5. Immediately after centrifugation carefully transfer the supernatant to a clean tube. Keep the supernatant!
6. Measure protein concentration with Bradford assay and adjust protein concentration to 3mg/ml.
7. Aliqupte and keep at -70°C.