Brain Nuclear Extracts

(2/97, AG; modified from David Frank)

Lysis Procedure

1. Place small piece of frozen ctx (approx one hemisphere of E18 Ctx) in 1 ml PBS lysis buffer.

2. Homogenize with dounce homogenizer (5 strokes with A, 5 strokes with B).

3. Transfer homogenate to eppendorff tube. Spin at 4 C for 5 mins.

4. Discard supernatant, resuspend pellet (nuclei) in 1 ml Extraction Lysis Buffer.

5. Sonicate nuclei with two 15 sec bursts.

6. Spin at 4 C for 15 mins.

7. Recover supernatant and transfer to new tube.

8. Quantify protein concentration by Bradford Assay. Label tubes and freeze at -70 C.

9. To run lysate in gel, suspend required amount in 2X SDS sample buffer (50 µl) with b-mercapto-ethanol, boil, cool on ice, and load.

Note: To make whole brain lysate, go directly to step 4 and start by placing tissue in 1 ml extraction buffer.

PBS Lysis Buffer (make fresh with cold PBS):
PBS 5 mls
200mM PMSF 25 µl
2mg/ml Aprotinin 25 µl

Extraction Buffer (for 100mls; store at 4 C):
ddH2O 83 mls
1M Tris (pH7.8) 2 mls (20 mM)
5M NaCl 2.5 mls (125 mM)
1M MgCl2 0.5 mls (5 mM)
0.5 M EDTA 40 µl (0.2 mM)
Glycerol 12 mls (12%)
NP40 100µl (0.1%)

Just before using prepare **Extraction Lysis Buffer** as follows:
Extraction buffer 5 mls
Aprotinin 25 µl
PMSF (200 mM) 25 µl
200 mM DTT 250 µl
Leupeptin (10 mg/ml) 5 µl