

# Nuclear and Cytoplasmic Extraction

Protocol Modified for Murine Cerebellum

NDJ

V.1

NE-PER Nuclear and Cytoplasmic Extraction Reagents. #78833 Pierce Biotech, Rockford, IL

**Throughout extraction keep tubes on ice and perform spin at 4°C**

**Table of NE-PER Reagents Amounts**

Tissue (mg)	CER I (ul)	CER II (ul)	NER (ul)
1	5	0.275	2.5
20	100	5.5	50
40	200	11	100
60	300	16.5	150

1. Remove cerebellum from mouse. Half cerebellum and separately freeze each cerebellar hemisphere.
2. Estimate mass of cerebellar hemispheres and add CER I (see table above for CER I, CER II, and NER amounts).
3. Liquify the tissue using motor-pestle. Same technique as preparing cerebellar tissue for Western Blotting.
4. Vortex for 15 seconds.
5. Incubate on ice(wet) for 10 minutes.
6. Add ice-cold CER II.
7. Vortex 5 seconds and put on ice for a minute.
8. Vortex 5 seconds.
9. Centrifuge 5 minutes (13000xg)
10. Transfer the supernatant (cytoplasmic extract) fraction to clean pre-chilled tube.
11. Spin cytoplasmic extract 2 minutes (13000xg). Transfer supernatant to new tube (cytoplasmic extract) and save.
12. Add 200ul ice-cold PBS to pellet (nuclear extract) from step 10.
13. Flick and invert tube with nuclear extract.
14. Centrifuge 5 minutes (13000xg).
15. Discard supernatant from nuclear extract.
16. Re-suspend nuclear extract in ice-cold NER.
17. Vortex 15 seconds and return to ice.
18. Vortex every 10 minutes for 15 seconds for 40 minutes.
19. Centrifuge 10 minutes (13000xg).
20. Transfer supernatant to pre-chilled tube (nuclear extract).
21. Store all extracts @-80°C.