Small Scale Nuclear and Cytoplasmic Extracts for Neurons

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Solutions and Materials

Buffer A
25mM Hepes ph 7.0
25 mM KCl
0.05 mM EDTA
5mM MgCl$_2$
10% glycerol
0.1% NP-40
1mM DTT (add this at the last minute from a freshly prepared stock)
+ protease inhibitors ( aprotinin, benzamidine, leupeptin, pepstatin, PMSF, or PMSF and
100x Protease inhibitor cocktail from Calbiochem, we use ‘Protease Arrest’),
phosphatase inhibitors: microcystein and 2µM cyclosporine A.

Buffer B
0.3 M Hepes ph 7.6
1.4 M KCl
30 mM MgCl$_2$
DTT 1mM
+ protease inhibitors ( aprotinin, benzamidine, leupeptin, pepstatin, PMSF, or PMSF and
100x Protease inhibitor cocktail from Calbiochem, we use ‘Protease Arrest’),
phosphatase inhibitors: Microcystein, β-glycerol phosphate (1M=100x stock), PNPP
(1M=50x stock), NaF (1M =20x stock) and 2µM cyclosporine A.

Buffer C
50mM Hepes ph 7.6
50 mM KCl
0.1 mM EDTA
1 mM DTT
Glycerol 10%
+ protease inhibitors ( aprotinin, benzamidine, leupeptin, pepstatin, PMSF, or PMSF and
100x Protease inhibitor cocktail from Calbiochem, we use ‘Protease Arrest’),
phosphatase inhibitors: Microcystein, β-glycerol phosphate (1M=100x stock), PNPP
(1M=50x stock), NaF (1M =20x stock) and 2µM cyclosporine A.

Ammonium Sulfate (NH$_4$)$_2$SO$_4$ 3 M (pH 7.9)
+ protease inhibitors ( aprotinin, benzamidine, leupeptin, pepstatin, PMSF, or PMSF and
100x Protease inhibitor cocktail from Calbiochem, we use ‘Protease Arrest’),
phosphatase inhibitors: microcystein and 2µM cyclosporine A.
It is critical that the extracts are prepared in a cold room and that all reagents, tips, tubes are prechilled.

1. Pre-cool the rotor, tubes and buffers in the cold room overnight.

**Centrifuge Tubes** This protocol was written for the 2 ml tubes for Beckman mini-ultra centrifuge TLA 100.2 tube, or polycarbonate 7/16 inch by 1 3/8 inch tubes (343770)

**Procedure**

**Cell Lysis** Be certain to add the protease inhibitors and freshly made DTT just before starting!

**For cultured neurons:**

1. Aspirate medium from plates at RT.

2. Gently and quickly rinse with RT PBS and aspirate the PBS off quickly and gently. Place plates on ice and add 1 ml of Buffer A (ice cold) on the plates. At this point transfer everything to the cold room.

3. Scrape the cells off the plates with a cell scraper and pipet the cells into an Eppendorf tube. Check for cell lysis by staining with trypan blue (if the lysis was complete you should see oval, dark blue nuclei and no intact cells, if it was incomplete one can see cells that either exclude trypan blue or are faintly stained).

4. Spin in eppendorf at a setting of 3 for 3 minutes.
   5a For cytoplasm save supernatants. Add 1/10th volume buffer B. Skip to step 9.
   5. add 1 ml of buffer A (no NP-40) and rinse the nuclei and spin in an Eppendorf centrifuge at a setting of 3 for 3 minutes and remove the supernatant.

6. Resuspend pelleted nuclei in 250 µl of buffer C; measure total volume and add additional buffer C to a total volume of 315 µl.

7. Add 35 µl 3 M ammonium sulfate to bring the final concentration to 0.3 M and rock for 30 minutes in the cold room.

8. The solution should become thick and viscous as the nuclei break and the nuclear proteins are released. Transfer to a TLA-100.2 ultracentrifuge tube. Since the solution is viscous you have to use a wide orifice tip or cut off the tip from a blue tip to transfer into the ultracentrifuge tube.

9. Spin 10-15 minutes at 100,000 rpm at 4 degrees C. To continue cytoplasmic prep skip to 11a.

10. Transfer 300 µl of the supernatant to a second TLA-100.2 tube.
11 Add an equal volume of 3 M ammonium sulfate. Pipette to mix.
   11a. For cytoplasm, remove 800 µl supernatant and add 0.3 g/ml (NH₄)₂SO₄ and
   rotate in the cold room until the Ammonium sulfate is completely dissolved

12. Spin 10 minutes at 100,000 rpm and 4 degrees C.

13. Remove the supernatant. Note: if you have scaled down this procedure and have a
   very small amount of protein, clear the wall of the tube with either a flame-polished
   Pasteur pipet or with a cotton plugged yellow pipet tip instead of running a spin column.
   The pellet from neurons is often found at the side of the tube or floating on the top. Be
   careful not to loose the pellet when removing the supernatant

14. Resuspend in 50-100 ul of Buffer C for cytoplasmic and 30-50 µl for nuclear extract.
   It is sometime hard to dissolve the pellet by just pipeting, in that case transfer into an
   Eppendorf tube and rotate in the cold room until it dissolves.

15. Freeze samples on dry ice and transfer into a -80C freezer.

For intact brains

1. quickly dissect brains and, place on on ice & cut in PBS in small pieces and transfer
   tissue into a Dounce Tissue Grinder. Gently and quickly aspirate the PBS off and add 2
   ml of Buffer A (ice cold) and aspirate off and replace with an adequate volume for the
   amount of tissue you are using.

2. Gently homogenize the tissue, but be careful to not break the nuclei.
   Check the nuclei with trypan blue (you should see oval, dark blue nuclei and no intact
   cells) and continue with the protocol as outlined above. Adjust the volume to the amount
   of tissue used.