Cell Culture Preparation

1. For adherent cells, harvest with 0.5mM EDTA and then centrifuge at 500×*g* for 5 minutes. For suspension cells, harvest by centrifuging at 500×*g* for 5 minutes.
2. Wash cells by suspending the cell pellet with PBS.
3. Transfer 1-10×10^6 cells to a 1.5mL microcentrifuge tube and pellet by centrifugation at 500×*g* for 2-3 minutes.
4. Use a pipette to carefully remove and discard the supernatant, leaving the cell pellet as dry as possible.
5. Add ice-cold CER I to the cell pellet (Table 1). Proceed to Cytoplasmic and Nuclear Protein Extraction, using the reagent volumes indicated in Table 1.

Table 1. Reagent volumes for different packed cell volumes.*

Packed Cell Volume (µL)	CER I (µL)	CER II (µL)	NER (µL)
10	100	5.5	50
20	200	11	100
50	500	27.5	250
100	1000	55	500

*For HeLa cells, 2×10^6 cells is equivalent to 20µL packed cell volume.

Tissue Preparation

1. Cut 20-100mg of tissue into small pieces and place in a microcentrifuge tube.
2. Wash tissue with PBS. Centrifuge tissue at 500×*g* for 5 minutes.
3. Using a pipette, carefully remove and discard the supernatant, leaving cell pellet as dry as possible.
4. Homogenize tissue using a Dounce homogenizer or a tissue grinder in the appropriate volume of CER I (Table 2).

Proceed Cytoplasmic and Nuclear Protein Extraction, using the reagent volumes indicated in Table 2.

Table 2. Reagent volumes for different tissue amounts.*

Tissue Weight (mg)	CER I (µL)	CER II (µL)	NER (µL)
20	200	11	100
40	400	22	200
80	800	44	400
100	1000	55	500

*Different tissue types may require more or less NE-PER Reagents per weight to optimally extract cytoplasmic and nuclear proteins.

Cytoplasmic and Nuclear Protein Extraction

Note: Scale this protocol depending on the cell pellet volume (Tables 1 and 2). Maintain the volume ratio of CER I:CER II : NER reagents at 200:11:100 µL, respectively.

1. Vortex the tube vigorously on the highest setting for 15 seconds to fully suspend the cell pellet. Incubate the tube on ice for 10 minutes.
2. Add ice-cold CER II to the tube.
3. Vortex the tube for 5 seconds on the highest setting. Incubate tube on ice for 1 minute.
4. Vortex the tube for 5 seconds on the highest setting. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge (~16,000×*g*).
5. Immediately transfer the supernatant (cytoplasmic extract) to a clean pre-chilled tube. Place this tube on ice until use or storage (see Step 10).
6. Suspend the insoluble (pellet) fraction produced in Step 4, which contains nuclei, in ice-cold NER.
7. Vortex on the highest setting for 15 seconds. Place the sample on ice and continue vortexing for 15 seconds every 10 minutes, for a total of 40 minutes.

8. Centrifuge the tube at maximum speed (~16,000×*g*) in a microcentrifuge for 10 minutes.
9. Immediately transfer the supernatant (nuclear extract) fraction to a clean pre-chilled tube. Place on ice.
10. Store extracts at -80°C until use.