



Nuclear & Cytoplasmic Extract Protocol

The protocol for the isolation of nuclear and cytoplasmic fractions can be used to generate material for the gel shift/gel super shift assays or any other assay requiring similar source material. Remember to use only specially prepared reagents that are free of any contaminating proteases or nucleases and to keep the preparation on ice where indicated. A common troubleshooting finding is that breakdown products caused by non-specific proteolysis are recognized in systems typically as lower molecular weight bands.

I. Reagents Required

Reagent	Preparation
Cells for Nuclear Extract	Prepare cells from a cell line known to be positive for the intended assay. For NFκB HeLa or Jurkat cells can be used.
Molecular Biology Grade Water	N/A
Phosphate Buffered Saline (PBS)	Use 10X PBS, pH 7.2 (0.2 M Potassium Phosphate, 1.5 M NaCl) (MB-008). Dilute appropriate volume to 1X with molecular biology grade water.
Cytoplasmic Extract (CE) Buffer with NP-40	Prepare a 1X solution composed of 10 mM HEPES, 60 mM KCl, 1 mM EDTA, 0.075% (v/v) NP-40, 1mM DTT and 1 mM PMSF, adjusted to pH 7.6. Convenient concentrated stocks of these reagents can be prepared such that 10 mL volumes of 1X CE buffer can be easily prepared.
CE Buffer without Detergent	Same as above except without NP-40.
Nuclear Extract (NE) Buffer	Prepare a 1X solution composed of 20 mM Tris Cl, 420 mM NaCl, 1.5 mM MgCl ₂ , 0.2 mM EDTA, 1mM PMSF and 25% (v/v) glycerol, adjusted to pH 8.0. Convenient concentrated stocks of these reagents can be prepared such that 10 mL volumes of 1X CE buffer can be easily prepared.
5 M NaCl Solution	Prepare a solution of sodium chloride at 5.0 M.
Glycerol	N/A

II. Procedure

1. Prepare approximately 4×10^8 cells per extract.
2. Wash the cells gently with PBS buffer. Collect the cells by centrifugation using a microcentrifuge at 1000 rpm.
3. Resuspend the pellet in 5 pellet volumes of CE buffer (approximately 100 μ L).
4. Incubate on ice for 3 minutes.
5. Spin the preparation using a microcentrifuge at 1000–1500 rpm for 4 minutes.
6. Remove the cytoplasmic extract from the pellet to a clean tube.
7. Wash the nuclei with 100 μ L of CE buffer without detergent. Be careful to resuspend the fragile nuclei gently.
8. Spin the nuclei as above at 1000–1500 rpms for 4 minutes.
9. Add 1 pellet volume NE buffer to nuclear pellet (approximately 50 μ L).
10. Adjust the salt concentration to 400 mM using 5 M NaCl (add ~35 μ L).
11. Add an additional pellet volume of NE buffer. Vortex to resuspend the pellet.
12. Incubate the extract on ice for 10 minutes. Vortex the mixture periodically to resuspend the pellet.
13. Spin the CE and NE at maximum speed for 10 minutes to pellet any nuclei.
14. Transfer the contents of the CE tube and NE tube separately to clean tubes. Add glycerol to the CE tube to 20%. Store at -70°C.

References

- Baldwin. 1996. Ann.Rev.Immunol.14: 649-681.