ROCKLAND™
antibodies & assays

Nuclear and 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.

Reagents Required

- Cells for Nuclear Extract. Prepare cells from a cell line known to be positive for the intended assay. For NFkB HeLa or Jurkat cells can be used.
- Molecular Biology Grade Water.
- PBS Wash Buffer. Phosphate Buffered Saline (PBS). Use 10X PBS, pH 7.2 (0.2 M Potassium Phosphate, 1.5 M NaCl) Code # MB-008. Dilute appropriate volume to 1X with molecular biology grade water.
- Cytoplasmic Extract (CE) Buffer with NP40. Prepare a 1X solution composed of 10 mM HEPES, 60 mM KCl, 1 mM EDTA, 0.075% (v/v) NP40, 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. Similar to above buffer but without the addition of NP40.
- Nuclear Extract (NE) Buffer. Prepare a 1X solution composed of 20 mM Tris Cl, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 1 mM 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.

Procedure

1. Prepare approximately 4 x 107 cells per extract.
2. Wash the cells gently with PBS buffer. Collect the cells by centrifugation using a microcentrifuge at 1000 rpms.
3. Resuspend the pellet in 5 pellet volumes of CE buffer (approximately 100 µl).
4. Incubate on ice for 3 min.
5. Spin the preparation using a microcentrifuge at 1000 to 1500 rpms for 4 min.
6. Remove the cytoplasmic extract from the pellet to a clean tube.
7. Wash the nuclei with 100 µl of CE buffer without detergent. Be careful to resuspend the fragile nuclei gently.
8. Spin the nuclei as above at 1000 to 1500 rpms for 4 min.
9. Add 1 pellet volume NE buffer to nuclear pellet (approximately 50 µl).
10. Adjust the salt concentration to 400 mM using 5 M NaCl (add ~35 µl).
11. Add an additional pellet volume of NE buffer. Vortex to resuspend the pellet.
12. Incubate the extract on ice for 10 min. Vortex the mixture periodically to re-suspend the pellet.
13. Spin the CE and NE at maximum speed for 10 min 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.