Preparation of nuclear extract using high salt and sonication

To extract nuclear proteins that are chromatin bound as well as histones.

1. Grow cells to desired density and amount and collect into tubes.
2. Resuspend the cell pellets using 4-5 pellet volumes of COLD buffer A supplemented with 0.1% NP40 and protease and phosphatase inhibitors and DTT if desired.
   a. Note: do not add DTT if an immunoprecipitation will be performed with this lysate
3. Pipet gently up and down.
4. Leave 20 min on ice with pipetting at 10min.
5. Spin 9800 x g for 10 minutes at 4°C.
6. Carefully remove and save the supernatant which is the cytosolic fraction, if desired. If saving, then aliquot a 50-100ul sample for protein concentration determination. Aliquot the remainder and snap- freeze and store at -80C.
7. Resuspend the pellet (intact nuclei) in ~1.5x more volume than the volume used in Step 2 of COLD buffer B supplemented with 700mM NaCl, protease inhibitors, phosphatase inhibitors and DTT to 1mM.
   a. For example, if 3mL of COLD Buffer A was used in Step 2, then use 4.5mL of COLD Buffer B.
   b. Note: do not add DTT if an immunoprecipitation will be performed with this lysate
8. Leave 10 minutes on ice. The solution may become viscous.
9. Sonicate in 10 sec pulses on ice until the solution become liquidly. We use an amplitude @ 65% and usually 5-6 cycles.
   a. The temperature should not go over 10°C during the sonication as proteins will denature.
10. Spin at 15,000 x g for 15 minutes to pellet insoluble material.
    a. Note: The pellet should be very small. If not, resuspend pellet and sonicate again.
11. Carefully remove the supernatant as the nuclear extract. Aliquot a 50-100ul sample for protein concentration determination and aliquot the remainder and snap-freeze and store at -80C.
12. Perform protein concentration determination.
13. **Note**: This extract can be dialyzed or diluted to reduce the salt concentration which may interfere with SDS-PAGE analysis.

Buffers

1. ***Buffer A***, 10 mM Hepes pH 7.9, 5 mM MgCl2, 0.25M Sucrose. This can be made ahead and stored frozen in aliquots. For complete formulation add NP40 to 0.1%, protease and phosphatase inhibitors and DTT if desired.
2. ***Buffer B***, 25 mM Hepes pH 7.9, 20%, glycerol, 1.5 mM MgCl2, 0.1 mM EDTA. This can be made ahead and stored frozen in aliquots. For complete Formulation, add NaCl to 700mM using powder, protease and phosphatase inhibitors and DTT if desired.

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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