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High Salt Nuclear Extract Preparation

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Protocol status: Working
We use this protocol and it's working

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Abstract

Adapted from Dignam JD, Lebovitz RM, Roeder RG. 1983. **Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei.** Nucleic Acids Res. 11(5):1475-89. PMID:6828386

Guidelines

The protocol is suitable for HeLa S3 cells. Parameters should be adapted for other cell types.

Before start

Prepare the following buffers and store at +4°C:

	PMSF 0.2 M: **3.48 g /100 ml DMSO**			
	TGME: **Glycerol** **Tris, pH 7.9** **$MgCl_2$** **EDTA** **water**	**Stock** **100 %** **0.5 M** **1 M** **0.5 M**	**Final Conc** **50 %** **50 mM** **5 mM** **0.1 mM**	**Vol (for 1 l)** **500 ml** **100 ml** **5 ml** **0.2 ml** **to 1 l**
	Low Salt Buffer: **Tris, pH 7.3** **Glycerol** **$MgCl_2$** **EDTA pH 8** **water**	**Stock** **1 M** **50 %** **1 M** **0.5 M**	**Final Conc** **20 mM** **12.5 %** **1.5 mM** **0.2 mM**	**Vol (for 1 l)** **20 ml** **250 ml** **1.5 ml** **0.4 ml** **to 1 l**
	High Salt Buffer: **Tris, pH 7.3** **Glycerol** **$MgCl_2$** **EDTA pH 8** **KCl** **water**	**Stock** **1 M** **50 %** **1 M** **0.5 M** **3 M**	**Final Conc** **20 mM** **12.5 %** **1.5 mM** **0.2 mM** **1.2 M**	**Vol (for 1 l)** **20 ml** **250 ml** **1.5 ml** **0.4 ml** **400 ml** **to 1 l**
	Hypotonic Buffer (HB): **Tris, pH 7.3** **KCl** **$MgCl_2$** **water**	**Stock** **1 M** **3 M** **1 M**	**Final Conc** **10 mM** **10 mM** **1.5 mM**	**Vol (for 1 l)** **10 ml** **3.34 ml** **1.5 ml** **to 1 l**
	10 x Buffer **Tris, pH 7.3** **KCl** **$MgCl_2$** **water**	**Stock** **1 M** **3 M** **1 M**	**Final Conc** **30 mM** **140 mM** **3 mM**	**Vol (for 1 l)** **30 ml** **46.6 ml** **3 ml** **to 1 l**

Cold room set up:
thaw 0.2 M PMSF
prepare hypotonic buffer (500 ml HB + 0.35 ml beta-ME + 0.5 ml 0.2 M PMSF)
prepare freezing solution (20 ml TGME + 0.4 ml DTT, in a 50 ml tube)
prepare tubes

Preparation of Cells

1 (Note: keep tubes on ice between centrifugations)
Pellet cells at 1500 x g for 8 mins in appropriate tube (eg 50 ml tubes)
Remove supernatant and wash cells once in PBS
Centrifuge at 1000 x g for 10 mins
Remove all supernatant and record the packed cell volume (PCV) in each tube
Fill tube with HB and carefully resuspend cells
Spin at 1000 x g for 5 mins
Verify that the swollen cell volume (SCV) is greater than PCV
Carefully pour off supernatant (pellet may be loose)
Add 2 x PCV volume of HB and carefully resuspend the cells
Place on ice for 10 mins

Cell homogenization

2 homogenize cells by douncing 15 times (for HeLa S3, to be determined for other cell types), check for lysis by microscopy
spin homogenized cells at 2600 x g for 15 mins
record nuclear pellet volume (NPV)
remove supernatant (cytoplasm)

Nuclear Extraction

3 calculate NPV/2 and add the amount of low salt buffer and high salt buffer to separate tubes
to each buffer add 0.0007 x NPV/2 of beta-ME, 0.01 x NPV/2 of 0.2 M PMSF, mix well
resuspend nuclear pellets in ½ low salt buffer and dounce 6 times
add the high salt buffer while mixing continuously
mix for 30 mins after the addition of all of the high salt buffer
spin at 15000 x g for 30 min
recover supernatant
for storage, snap freeze in liquid N_2 and keep at -80°C