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High Salt Nuclear Extract Preparation

PLOS Pathogens

Xavier Contreras¹, Rosemary Kiernan¹

¹Institut de Genetique Humaine, UMR9002, Montpellier, France

Rosemary Kiernan

Institut de Genetique Humaine, Montpellier, France

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.

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

BEFORE START INSTRUCTIONS

Created: Oct 30, 2017

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

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PROTOCOL integer ID:
8474

Keywords: nuclear extract,
Dignam, transcription

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

- (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 N₂ and keep at -80°C