High Salt Nuclear Extract Preparation

Xavier Contreras,Rosemary Kiernan

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

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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:	**Stock**	**Final Conc**	**Vol (for 1 l)**
Glycerol	100 %	50 %	500 ml
Tris, pH 7.9	0.5 M	50 mM	100 ml
$MgCl_2$	1 M	5 mM	5 ml
EDTA	0.5 M	0.1 mM	0.2 ml
water			to 1 l

Low Salt Buffer:	**Stock**	**Final Conc**	**Vol (for 1 l)**
Tris, pH 7.3	1 M	20 mM	20 ml
Glycerol	50 %	12.5 %	250 ml
$MgCl_2$	1 M	1.5 mM	1.5 ml
EDTA pH 8	0.5 M	0.2 mM	0.4 ml
water			to 1 l

High Salt Buffer:	**Stock**	**Final Conc**	**Vol (for 1 l)**
Tris, pH 7.3	1 M	20 mM	20 ml
Glycerol	50 %	12.5 %	250 ml
$MgCl_2$	1 M	1.5 mM	1.5 ml
EDTA pH 8	0.5 M	0.2 mM	0.4 ml
KCl	3 M	1.2 M	400 ml
water			to 1 l

Hypotonic Buffer (HB):	**Stock**	**Final Conc**	**Vol (for 1 l)**
Tris, pH 7.3	1 M	10 mM	10 ml
KCl	3 M	10 mM	3.34 ml
$MgCl_2$	1 M	1.5 mM	1.5 ml
water			to 1 l

10 x Buffer	**Stock**	**Final Conc**	**Vol (for 1 l)**
Tris, pH 7.3	1 M	30 mM	30 ml
KCl	3 M	140 mM	46.6 ml
$MgCl_2$	1 M	3 mM	3 ml
water			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

Protocol

Preparation of Cells

Step 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

Step 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

Step 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