

DNase I Hypersensitivity Assay

2-4 T150's

- 1) Harvest the cells by trypsinization, and transfer to 15ml conical flask
- 2) Pellet the cells by centrifugation at 800 rpm for 6 minutes.
- 3) Wash the pellet with PBS (1X) count cells and determine total number of cells _____
- 4) Pellet cells.
- 5) Resuspend cells in RBS ~3ml/T150

RBS (Nuclei Isolation Buffer)

10 mM Tris, pH 7.4
5 mM MgCl₂
0.5 mM DTT* add fresh
0.3 mM sucrose (DNase/RNase free)

- 6) Add 1/100 –1/200 volume of 10% NP-40 on ice.
- 7) Dounce in a sterile homogenizer, 10 strokes.
- 8) Check for lysis and count nuclei (optional) with 1:1 (homogenate and .4% trypan blue).
- 9) Spin 1500rpm 5 min.
- 10) Resuspend nuclei in _____ml RBS (without NP-40). Want a final concentration of $>10^7$ cells/ml and at least 1.8 mls total. Keep consistent within and between runs.
- 11) Immediately add 200 uL of nuclei ($>2 \times 10^6$) _____ cells to already prepared aliquots DNase I. 50ul aliquots of DNase I (with 2.5 ul of 10mM CaCl₂) in 8, 1.5 ml eppendorfs. Mix by inversion.

Incubate 10 min at 22 C.

DNase: 0U, 2 .5U, 5U , 10U, 20U, 40U

two controls: Nuclei extract with CaCl₂ w/o DNaseI (endogenous nuclease control) OU

Nuclei extract only kepted on ice (nuclease free reference)

- 12) After incubations stop reactions with 250ul stop solution at 50 C overnight.

Stop Solution

20mM EDTA
1%SDS
1mg proteinase K/ml * add fresh

- 13) Add 50 ul 10M NH₄OAc
- 14) Extract twice with equal volume (550ul) P:C:I, Phenol : Chloroform : Isoamyl Alcohol 25 : 24 : 1, equilibrated to pH 8 with Tris/EDTA.

Mix by inversion. Never vortex.

Spin in microfuge at max speed.

Transfer the aqueous phase (top) to a new eppendorf. Repeat extraction.

- 15) Repeat extraction with C: I.
- 16) Add 50ul 10 M NH₄OAc and 1.1ml 100% EtOH (at least 2 volume)
- 17) Precipitate over night at -20 C.
- 18) Spin 17k in cold room for 30 min.
- 19) Wash pellet with 70% EtOH
- 20) Resuspend in 20 ul LoTE

P : C : I (25 : 24 : 1, store at 5 °C, protect from light with foil)

Melt phenol at 65°C (hydroxyquinoline already added)

Add an equal volume of C : I (24 : 1). Mix.

Add equal volume 0.5M Tris pH 8. Shake, allow phases to separate at 5 °C o/n.

Draw of Tris, add equal vol TE pH8, Shake, allow phases to separate at 5 °C. Repeat.

Remove aqueous and overlay with TE.

TE

10mM Tris pH8

1mM EDTA

Low TE

0.1 X TE

C:I

Mix chloroform: isoamylalchol 24:1

Overlay with TE pH 8 shake and allow phases to separate. Store at 5 °C.