epiVAR_BP_DNase
Version: BluePrint

Stock solutions

Deionized IGEPAL CA-630

Warm stock bottle of IGEPAL CA-630 to 37°C as it is quite viscous at room temperature. Make a 10% solution by adding 4mL of warmed IGEPAL (dispensed via a 5mL syringe attached to an 18 gauge needle) to 36mL of Milli-Q or Molecular Biology Grade sterile dH20. Vortex extensively until solubilized.

Add 2 grams AG501-X8 resin to the 40mL 10% IGEPAL solution. This resin will "deionize" the solution. "Spent" resin will be golden in colour; the solution is still deionized if the resin is a combination of blue and golden beads.

Store solution in a tinfoil-wrapped conical tube at 4°C.

0.5M Spermine

Dissolve 5 grams Spermine Free Base in 49.43mL final volume Milli-Q or Molecular Biology Grade sterile dH20.
Store in convenient aliquots at -20°C.

0.5M Spermidine

Dissolve 1 gram Spermidine Free Base in 13.77mL final volume Milli-Q or Molecular Biology Grade sterile dH20.
Store at 4°C.

DNaseI 10X Digestion Buffer (per 50mL)

Final concentration	Stock concentration	Amount used from stock
60mM CaCl2	1M CaCl2	3mL
750mM NaCl	5M NaCl	7.5mL

Combine stock solutions and 39.5mL Milli-Q or Molecular Biology Grade sterile dH20. Can be stored at room temperature up to 1 year.

Stock DNaseI

Solubilize on ice with no vortexing an entire bottle of DNaseI Type II from Bovine Pancreas in the following storage buffer at a final concentration of 10U/µL:

20mM Tris-HCl, pH 7.6
50mM NaCl
2mM MgCl2
2mM CaCl2
1mM Dithioerythritol
0.1 mg/mL Pefabloc SC
50% Glycerol

Store in 250 µL aliquots at -20°C.

Stop Buffer (per Litre)

Final concentration	Stock concentration	Amount used from stock
50mM Tris-HCl, pH 8.	0 1.0M Tris-HCl, pH 8.0	50mL
100mM NaCl	5.0M NaCl	20mL
0.10% SDS	10% SDS	10mL
100mM EDTA, pH 8.0	0.5M EDTA, pH 8.0	200mL
Molecular Biology Grade sterile H2O		720mL

Combine stock solutions and add sterile dH2O to a final volume of 1 liter. Dispense into 25mL aliquots and store at 4°C. (SDS will precipitate upon storage at 4°C but will go back into solution upon warming to 37°C).

On day of experiment

ON ICE:

Buffer A (per Liter)

Final Concentration	Stock concentration	Amount used from stock
Sterile MilliQ Water		230mL
15mM Tris-HCl, pH 8.0	1M Tris-HCl, pH 8.0	3.75mL
15mM NaCl	5M NaCl	750ul
60mM KCl	1M KCl	15mL
1mM EDTA, pH 8.0	0.5M EDTA, pH 8.0	0.5mL
0.5mM EGTA, pH 8.0	0.5M EGTA, pH 8.0	250ul
0.5mM Spermidine	0.5M Spermidine Free Base	250ul

Combine indicated amounts of stock solutions and sterile dH2O to a final volume of 250ml. Store at 4°C. Use within 1 week.

Buffer A + PIC
1 sample - Add 1 X PIC aliquot (600ul) to 14.4 ml of Buffer A
2 samples - Add 2 X PIC aliquot (1200ul) to 28.8 ml of Buffer A

2X IGEPAL CA-630 Solution
Add 15µL 10% IGEPAL CA-630 to 5mL of Buffer A+PIC

AT 37oC

1X DNaseI Digestion Buffer
In 15ml falcon add 500ul 10X DNaseI Digestion Buffer to 4.5mL Buffer A+PIC.

Stop Buffer + Spermidine and Spermine
In a 15ml falcon:

5ml	Stop Buffer
10µl	0.5M Spermidine Free Base
3µl	0.5M Spermine Free Base
25µl	Proteinase K

Protocol - Use 5 million cells

1. In a 2ml eppendorf add 5 million cells.
2. Add Buffer A+PIC to 2ml
3. centrifuge for 5 minutes at 1,500 x g (4,000 rpm on Sorval Fresco) 4°C
4. Add 1ml of Buffer A + PIC and centrifuge for 5 min at 1,500 x g, 4°C
5. Pipette off supernatant and resuspend gently in 1ml of Buffer A + PIC
6. Add 1ml of 2X IGEPAL CA-630 solution (0.03%).
7. Mix well by inversion and incubate on ice 5-6 minutes.
8. Pellet nuclei for 5 minutes at 1,500 x g, 4°C
9. Carefully aspirate supernatant, as pellet is quite “slippery.”
10. Resuspend cells in 500ul of ice-cold Buffer A+PIC .
11. Aliquot cells into **3 X 1.5 ML tubes**

0U	100ul
60U	200ul
80U	200ul

12. Centrifuge for 5 minutes at 1,500 x g, 4°C
13. Aspirate supernatant from all nuclei pellets and place tubes in the water bath at 37°C

DNaseI Treatment of Hematopoietic Nuclei

1. To three 15 ml falcons add 0.6 ml 1X DNaseI Digestion Buffer

2 samples – add 1.2ml 1X DNaseI Digestion Buffer

2. Add appropriate amount of DNAse1 enzyme to each tube:

0U	-	
60U	3.6ul DNAse1	(2 samples - 7.2ul)
80U	4.8ul DNAse1	(2 samples – 9.6 ul)

   Mix thoroughly but gently by pipetting (DO NOT VORTEX) as the enzyme denatures easily with aeration.

3. Gently resuspend nuclei with 500ul of 1X DNaseI Digestion Buffer plus enzyme and without enzyme for undigested sample. Pipet 3 times gently using wide-bore tips to ensure homogenous suspension.

4. Incubate for 3 minutes at 37°C in water bath.

5. Add 500ul of Stop Buffer to DNaseI reaction tube and mix by Pipet 3 times.

6. When all tubes are complete transfer tubes to 55oC block (Program1)

7. Digest sample 1 hr in the 55°C / Overnight on block

8. Store treated samples at 4°C