

Hot-phenol-SDS RNA prep used in Kustu lab.

After [J Bacteriol 1993 Apr;175\(7\):2026-36](#).

Reagents, Solutions, etc

Phenol: Buffer-saturated Phenol, GibcoBRL, Cat. # 15513-039

Ethanol: Rossville, Gold Shield Ethanol (200 Proof)

Cell-Stop Solution: 5% Phenol in Ethanol (100%)- by volume

Cell Resuspension Buffer (4°C): 10mM KCl

5mM MgCl

10mM TrisCl (pH7.5)

Hot Phenol Buffer (RT): 0.4M NaCl

40mM EDTA

1% -Mercaptoethanol

1% SDS

20mM TrisCl (pH7.5)

Hot Phenol Solution: 5 parts Hot Phenol Buffer, 1 part Phenol

(Usually 400µL buffer, 80µL phenol per tube)

DNase I buffer (10x): 400mM TrisCl (pH7.5)

60mM MgCl₂

DNase I: Amersham Pharmacia Biotech, 10,000U/mL, RNase-free, 27-0514-01

Harvest

Grow Cultures to OD₆₀₀=0.4

Remove from H₂O bath, pipet quickly into Blue cap Falcon tubes with 1/10 volume of 5%

Phenol in 100% EtOH

(I usually harvested 2x 25mL into 2x 2.5mL Phenol/EtOH soltn.)

Invert rapidly to mix

Spin 5min, 4°C, 5500 x g

Freeze pellet (dry ice, then -80°C) or Extract RNA

RNA Extraction (for 25mL cells, harvested at OD₆₀₀=0.4) Wear **Gloves** from now on.

Defrost pellet in ice

Resuspend pellet in 960µL ice-cold cell resuspension buffer (4°C) by vortexing ~15-30s

Split sample (480µL each) into 2 tubes (1.5mL Epp), each with an equal volume (480µL)

of hot phenol solution (400µL buffer, 80µL phenol)

Vortex 15-30s

95°C, 1min

Spin down cell debris- Eppendorf microcentrifuge, 15min, 16000 x g, RT

Transfer Supernatant to 2mL Epp tubes

Extract with 600µL phenol/chloroform (1:1) by vortexing 10s, spin 1min in the Fisher

Extract **twice** with 600µL chloroform

RNA Precipitation

Add 900 μ L isopropanol, invert to mix, ice 10min

Spin 15 min, full-speed, RT

(Pellet should be translucent/white; not huge, but visible)

Wash pellet with 1mL 70% EtOH (-20°C) by vortexing 15s, spin 10min full-speed in cold room, pour out EtOH

Remove all EtOH by spinning 5s at RT, pipette out remaining EtOH, dry pellet at 42°C, ~3-5min

Resuspend RNA in 45 μ L H₂O by vortexing 5s, 37°C heatblock 5min, vortex 5s, spin down 5s

Combine like tubes (90 μ L total), keep on ice

Check RNA Concentration: OD₂₆₀ of 1/200 dilution- should have ~100-300 μ g RNA

DNase I

Add 10 μ L DNase I buffer (10x)- 4°C

1 μ L DNase I (10U)- this gives 100 μ L total volume

Tap tube to mix, spin down 5s

37°C heatblock, 30min

Add 10 μ L Sodium Acetate (3M, pH5.2), pipette to mix

Extract with 100 μ L phenol (vortex 10s, spin 1min)

Extract with 100 μ L phenol/chloroform (1:1)

Extract **twice** with 100 μ L chloroform

Precipitate RNA with 300 μ L 100% EtOH (-20°C), -20°C, 10min

Spin 15min, full-speed, 4°C, pour out EtOH

Wash pellet with 200 μ L 70% EtOH (-20°C)- vortex 15s, spin 10min, full-speed in cold room, pour out EtOH

Remove all EtOH: spin 5s, RT, pipette out remaining EtOH, dry pellet at 42°C, ~3-5min

Resuspend RNA in H₂O (usually 60 μ L or less, depending on RNA yield)- want ~3 μ g/ μ L

Check RNA Concentration- OD₂₆₀ of 1/200 dilution (lose ~20-40% after DNase I)

Store RNA at -80°C