

RNA Isolation from Yeast Cells

updated 12/28/04

To cell pellet, add **600 μ L 1X High Salt Buffer** and **600 μ L phenol/chloroform**. (25:24:1 Rouché #101003) or (25:24:1 Fisher BP 17521400) [use phenol/chloroform in fume hood]

Vortex 1min.

Incubate tubes @ **65°C, 4min**, followed by **ice 4min**.

Transfer solution to **2.0mL phase lock gel tube** (eppendorf #955154045), **spin @ 14K rpm, 5min**.

Transfer supernatant to 2.0mL microfuge tube. Add **1400 μ L 100% EtOH** and **incubate @ -80°C \geq 1hr to O/N**.

Pellet RNA by **centrifuging @ 14K rpm, 4°C for 30min**.

Remove EtOH and **resuspend pellet in RNase free water**.

$$[\text{RNA}] = \frac{(A_{260})(40\mu\text{g})(\text{d.f.})}{1000\mu\text{L}}$$

1X High Salt Buffer*	Stock	for 1mL
0.3M NaCl	5M NaCl (16.6X)	60 μ L
20mM Tris pH 7.6	1M Tris (50X)	20 μ L
10mM EDTA	0.5M EDTA (50X)	20 μ L
1% SDS	10% SDS (10X)	<u>100μL</u>
	RNase Free H ₂ O	800 μ L

* use only RNase free components to make high salt buffer.