

Phenol/Chloroform Extraction and Ethanol Precipitation

Note: Work in fume hood!

1. Add 1 Vol. saturated phenol (colorless!), vortex 30s and centrifuge 1 min full speed
2. Carefully remove supernatant (water phase) into new tube
3. Add 1 Vol. chloroform to supernatant, vortex 30s and centrifuge 1 min full speed
4. Remove supernatant (water phase) into new tube
5. If RNA concentration is less than 1 mM, add glycogen (~ 1 mg/mL final; stock 20 mg/mL)
6. Add 1/3 Vol. 1M KOAc pH 4.5
7. Add 2.5 Vol. cold ethanol
8. Incubate overnight at -20°C
9. Centrifuge at 4°C, full speed (13,000 rpm), 30 min
10. Resuspend pellet in H₂O (20 mL)