

BUFFERS AND SOLUTIONS

**!! Use RNase free ddH₂O to make buffers and solutions. DEPC-treat glassware.
!! Before starting, pre-cool buffers and reagents on ice, pre-cool centrifuges.**

- DEPC-water: (RNase free ddH₂O) Add 100 µl diethylpyrocarbonate (DEPC) to 1L of ddH₂O. Keep overnight at 37°C. Autoclave for at least 30 min. Or better: use commercial RNAs-free water
- RNA-extraction buffer (pH 7.5):
 - 0.1 M Tris-Cl
 - 1 mM EDTA
 - 0.1 M LiCl
- PCI:
 - 50% citrate-buffered and water equilibrated-phenol (pH 4.2)
 - 48% chloroform
 - 2% isoamylalcohol
- 10% or 20 %sodium dodecylsulfate (SDS)
- PCI with 1% (SDS)
- 40% potassium acetate (KAc) pH 5.5
- 100% and 70% ethanol

PROCEDURE

!! Always use RNase free ddH₂O. Wear gloves and change them regularly. Use RNase free labware (eppendorf tubes, glass beads, ...). Cool all buffers and solutions thoroughly on ice before use; keep 100% and 70% ethanol at -20 °C. ALWAYS KEEP SAMPLES ON ICE; use centrifuges at 2 °C or in the COLDROOM.

Day 1

Grow cultures.

Day 2

1. The cultures are cooled fast by adding 15-20 ml of a 50 ml culture to 30-40 ml of ice-cold water (in 50 ml Falcontube). Use larger volume of culture when working with slow growing or flocculating cells. Place on ice immediately, keep cold and process immediately.
2. Pellet cells (3000 rpm, 3 min) and remove supernatant. Resuspend cells in 1 ml of ice-cold water and transfer to a 2 ml Eppendorf tube (preferably safe-lock or screw-cap tube).

3. Pellet cells (3000 rpm, 3 min) and remove supernatant. Freeze immediately at -80°C . Ideally, steps 1-3 are performed fast and should take no longer than 10 min. The pellet size should be equivalent to $\pm 100\ \mu\text{l}$ of cells.

Day 3

4. Add 500 μl of acid washed glass beads (diameter 425-600 microns) to the frozen pellet. Use a 500 μl tube as a "bucket" to add the glass beads.
5. Add 500 μl PCI with 1% SDS and 500 μl RNA-extraction buffer, in this order.
6. Mix thoroughly for 20-30 min using a multitube vortex in the coldroom. The pellet should have thawed to assure optimal mixing. *Optional: to improve mixing and RNA-extraction, also vortex tubes individually for 30-60 sec in the coldroom.*
7. Spin (7000 rpm, 10 min) and transfer supernatant (aqueous phase) to a new 1.5 ml Eppendorf tube. *Optional: transfer to "Phase Lock Gel Tube, Heavy" (Eppendorf).* Make sure not to transfer the white, milky interfase.
8. Add 500 μl PCI, vortex thoroughly and spin (7000 rpm, 10 min). Transfer aqueous phase to a new 1.5 ml Eppendorf tube.
9. Add 25 μl 40% KAc and 1 ml 100% ethanol, invert tube several times and freeze at -80°C for a few hours or overnight.

(Day 4)

10. Defrost samples on ice and precipitate RNA by spinning (14000 rpm, 10 min). Remove ethanol with pipette.
11. Wash the precipitate with 700 μl 70% ethanol and spin (14000 rpm, 5 min). Remove ethanol carefully by pipette and remove remainder of ethanol by aspiration. Dry precipitate in the hood for at least 1 hour (but no longer than 2 hours) while keeping samples on ice.
12. Add 40-100 μl RNase free water, depending on the amount of isolated RNA. *Optional: add 1 μl RNase inhibitor to 100 μl of water before adding to tube.* To dissolve RNA, place tube with added water in the cold room for 1 hour and follow by resuspending precipitate.
13. Determine RNA concentration by measuring OD_{260} and OD_{280} ($\text{OD}_{260}/\text{OD}_{280} \approx 2$). Dilute 1 μl sample in 500 μl . Check for degradation on 1.2% agarose TAE formaldehyde gel. Use 1.33 g of agarose for 150 ml, add 3 ml of formaldehyde just before pouring gel. Use ddH₂O for gel and buffer. Run at 80 V. If no degradation has occurred you will see 3 bands, 2 for ribosomal RNA and one for mRNA.