

Fast Culture Sampling Protocol (submitted by Martin Kos, Tollervey lab)

Method developed specifically for the RiboSys project.

Grow cells in appropriate conditions to a required OD (for pulse chases ~ 0.4-0.5 in SD media; sterilize the media only by filtration).

1. Take 1 ml samples and pipette it directly into 10ml 100% ethanol in a 15 ml falcon tube on dry ice (dry ice/ethanol bath is also possible but can be messy).
2. Repeat in required time points
3. Transfer the tubes with frozen samples carefully on wet ice and let them warm up to 0 °C for several minutes. **DO NOT CLOSE THE LID** – the pressure changes due to the temperature change and the lids can pop – this is particularly important when working with radioactive samples. In this case I only gently lie the lids on top of the tubes
4. Close the lids tightly and invert the tubes couple times to mix
5. Centrifuge the cells at 3000g for 5 min at 4 °C.
6. Resuspend the cells in 1ml H₂O, transfer to an Eppendorf tube and spin
7. Gently decant the liquid and remove the remainder with a pipette (you can re-spin briefly)
8. Resuspend the pellet directly in 100 µl GTC/phenol mix and transfer in an Eppendorf tube (if no wash step) with 100 µl glass/zirconia beads
9. Proceed as usual with the small scale RNA extraction protocol

Notes

- When working with radioactivity (³H pulse-chase etc), it is almost impossible to avoid contamination of the centrifuge from falcon tubes. Therefore, after spin remove the centrifuge buckets and rinse them thoroughly – this is usually sufficient. Use Safe-Lock Eppendorf Tubes or Starlab or equivalent – this completely prevents contamination of small centrifuges!
- Expected yield: 10 - 20 µg of total RNA from 0.5 OD of cells in SD media.
- The whole experiment can be also performed in 2 ml tubes. Use 700 µl EtOH on dry ice and add 700 µl culture. As the EtOH “bubbles” a lot, it is important to check for contamination of centrifuges and blocks. However, it speeds up the whole protocol significantly.

Fast Culture Sampling Protocol – Larger volumes. (as used by Beggs lab for mRNA extraction).

Grow cells in appropriate conditions to a required OD (for Doxycycline inductions / repression ~ 0.4-0.7 in SC media).

1. Take 10 ml samples and pipette it directly into 5ml 100% ethanol in a 15 ml falcon tube on dry ice (final volume 33% ethanol – cools sample to approx -10°C). Sample may foam so gentle pipetting.
 2. Close lids gently and invert a couple of times to mix.
 3. Repeat in required time points
 4. After two minutes transfer the tubes on dry ice to wet ice and let them warm up to 0°C for several minutes. Close the lids tightly and invert the tubes couple times to mix
 5. Centrifuge the cells at 3000g for 5 min at 4°C.
 6. WASH STEP: resuspend cells in 1ml cold H₂O, transfer to an Eppendorf tube and spin
 7. Gently decant the liquid and remove the remainder with a pipette (you can re-spin briefly)
 8. Resuspend the pellet directly in 100 µl GTC/phenol mix (containing LSM T7 spike) with 100 µl glass/zirconia beads.
 9. Proceed as usual with the small scale RNA extraction protocol
- If there is still a large precipitate after final ethanol precipitation of RNA – substitute ammonium acetate for sodium acetate.
 - Expected yield: 200 - 300 µg of total RNA from 0.5 OD of cells in SC media.