Standard protocol for purification of 70S ribosomes[1]

Cells were grown for ~ 14 h at 37 °C to an OD600 of 1, harvested, washed once with saline, and stored at − 80 °C. The frozen pellet was thawed and resuspended in 20 mM K-HEPES pH 7.5, 100 mM NH4Cl, 10.5 mM Mg(OAc)2, 0.5 mM EDTA pH 8, 6 mM 2-mercaptoethanol, 0.5 mM PMSF. Cells were then mixed by inverting continuously at 37 °C for ~ 1 h and lysed by sonication (50% amplitude; 4 min Å~ 2; 5 s pulse on and 30 s pulse off) while on ice. Aliquots were taken pre-lysis and post-lysis to follow CFU/ml reduction. The cell lysate was clarified by centrifugation at 20,000 rpm for 20 min at 4 °C using a Beckmann Type 45 Ti rotor and a Beckman L8-70 M ultracentrifuge. Supernatant was loaded onto a sucrose cushion (20 mM K-HEPES pH 7.5, 1.1 M sucrose, 0.5 M KCl, 10.5 mM Mg(OAc)2, 0.5 mM EDTA pH 8) and centrifuged at 40,000 rpm for 21 h using a Beckman Type 45 Ti rotor. The ribosomal pellet was resuspended in 20 mM Tris-OAc pH 7.5, 10 mM MgOAc2, 400 mM KCl, 1.2 M (NH4)2SO4 at 4 °C, sterilized using a syringe filter (0.22 micron), and flash frozen at − 80 °C. All glassware was pre-treated with 0.1% DEPC sterilized water and autoclaved.
Crude ribosomes were diluted in buffer C containing 20 mM Tris-OAc, pH 7.5, 400 mM KCl, 10 mM Mg(OAc)2, 1.2 M (NH4)2SO4, and loaded onto a Toyopearl butyl 650S column. Using a reverse gradient of the same buffer without (NH4)2SO4, 70S peak fractions were pooled and diluted in buffer E (10 mM K-HEPES, pH 7.5, 50 mM KCl, 10 mM MgOac2, and 10 mM NH4Cl). Ribosomes were pelleted overnight in a Ti-45 rotor at 42,000 rpm for 21 h at 4 °C. Pellets were dissolved in buffer E with 8% sucrose, loaded onto a 12 to 40% sucrose gradient, and centrifuged in a Ti-15 zonal rotor at 27,000 rpm for 21 h at 4 °C. 70S peak fractions were pooled and diluted in buffer E without sucrose and pelleted as above. Ribosomes were resuspended in buffer G (5 mM K-HEPES pH 7.5, 50 mM KCl, 10 mM Mg(OAc)2, and 10 mM NH4Cl), concentrated using an Amicon Ultracel concentrator (30 kDa molecular weight cutoff) to a concentration of 8.9 µM, and flash-frozen at − 80 °C.

1. Murphy EL, Singh KV, Avila B, Kleffmann T, Gregory ST, Murray BE, Krause KL, Khayat R, Jogl G. Cryo-electron microscopy structure of the 70S ribosome from Enterococcus faecalis. Sci Rep. 2020;10(1):16301. Epub 2020/10/03. doi: 10.1038/s41598-020-73199-6. PubMed PMID: 33004869; PMCID: PMC7530986.