

Ribosome Production and Purification Protocol

1. Overnight Culture

1. Prepare 20 mL LB medium supplemented with 20 μ L of 50 mg/mL Kanamycin in a 100 mL Erlenmeyer flask (Final Kanamycin concentration = 50 μ g/mL).
 2. Using a sterile inoculation loop, streak a small amount of E. coli JE28 glycerol stock into the culture.
 3. Incubate at 37 °C, 260 rpm overnight.
-

2. Main Culture

1. Prepare 1 L LB medium supplemented with 1 mL of 50 mg/mL Kanamycin in a 2 L baffled flask (Final Kanamycin concentration = 50 μ g/mL).
 2. Inoculate with 10 mL of the overnight culture.
 3. Incubate at 37 °C, 260 rpm for 3–4 h.
 4. Harvest cells by centrifugation at 3,220 \times g, 4 °C for 10 min.
 5. Discard the supernatant and store the cell pellet at –80 °C.
-

3. Ribosome Purification

Buffer Preparation

Component	Lysis Buffer	Elution Buffer	Ribosome Buffer
Tris-HCl	20 mM, pH 7.6	20 mM, pH 7.6	–
HEPES	–	–	20 mM
MgCl ₂	10 mM	10 mM	–
Magnesium Acetate	–	–	6 mM
KCl	150 mM	150 mM	30 mM
NH ₄ Cl	30 mM	30 mM	–
Imidazole	–	150 mM	–
TCEP	–	–	1 mM (add fresh just before use)

Column Preparation

1. Add Sepharose resin (stored in 20% ethanol) to the chromatography column until the resin height reaches 3 cm.
2. Wash the column with 50 mL Milli-Q water to remove ethanol.
3. Equilibrate the column with 30 mL lysis buffer.

Cell Lysis

4. Resuspend the frozen cell pellet in 10 mL lysis buffer.
5. Sonicate on ice using a large probe:
 - 70% amplitude
 - 20 s ON / 20 s OFF
 - Total ON time: 3 min 40 s (11 cycles)
 - Keep the tip ~2 cm above the tube bottom; avoid touching tube sides.
6. Transfer lysate into 2 mL tubes and centrifuge at 21,130 × g (max speed) for 20 min at 4 °C.
7. Collect the supernatant and apply it to the column. Discard the pellet.

Column Wash & Elution

8. Wash the column with the following buffers:

Step	Buffer
Wash 1	30 mL of lysis buffer
Wash 2	30 mL of 5 mM imidazole buffer (29 mL lysis buffer + 1 mL elution buffer)
Wash 3	60 mL of 25 mM imidazole buffer (50 mL lysis buffer + 10 mL elution buffer)
Wash 4	30 mL of 40 mM imidazole buffer (22 mL lysis buffer + 8 mL elution buffer)
Wash 5	30 mL of 60 mM imidazole buffer (18 mL lysis buffer + 12 mL elution buffer)

10. Elute ribosomes with 7.5 mL elution buffer. Collect into a clean 15 mL tube.
-

4. Buffer Exchange

1. Transfer eluate into a 15 mL centrifugal filter (3 kDa MWCO) and centrifuge at 4,000 × g, 4 °C for ~60 min, concentrating to ~1 mL.
 2. Add 15 mL ribosome buffer to the filter and concentrate again to ~1 mL.
 3. Repeat the previous step one more time.
 4. Final volume after the last spin should be ~1 mL.
 5. Transfer to a low-protein-binding tube.
 6. Store at -80 °C.
-

5. Ribosome Concentration Measurement (Using NanoDrop)

1. Dilute the sample 1:100 in ribosome buffer.
2. Use ribosome buffer as blank.
3. Load 1 μL of diluted sample and measure absorbance at 260 nm.
 - A₂₆₀ of 10 (diluted sample) corresponds to 23 pM ribosomes in the original undiluted sample.
 - For in vitro translation, adjust concentration to 3.45 pM.

To adjust concentration:

- Dilute with ribosome buffer or
- Concentrate using a 3 kDa, 0.5 mL centrifugal filter at 14,000 \times g, 4 °C.