

Protocol: In vitro Reconstitution of 30S subunits from TP30 and 16S rRNA transcripts synthesized using T7 RNA Polymerase

(Reference: Krzyzosiak et al., and Ofengand. *Biochemistry* (1987) 26, 2353-2364;
Newcomb and Noller. *Biochemistry* (1999) 38, 945-951.)

1. Resuspend the purified 16S rRNA pellet in Buffer A. Incubate at 37 °C for 15 min.
2. Combine 60 pmoles of 16S rRNA transcript and 1 molar equiv of TP30 in Buffer A in 100 µl final volume.
3. Incubate sequentially for 15 min at each of the temperatures 40, 43, 46, 48, and 50 °C. Cool quickly to 4 °C (Use PCR machine to program the cycle).
4. Use the reaction mixture for subunit association:

Protocol: Subunit Association and Purification of 70S Ribosomes

(Reference: Newcomb and Noller. *Biochemistry* (1999) 38, 945-951.)

1. Incubate 100 µl of reconstitution reaction (above) with 6-12 pmol of 50S subunits in Buffer B at 37 °C for 30 min.
2. Layer the samples on 10-40% sucrose gradients prepared in buffer C. (This procedure is for 11 ml gradients and SW41 rotor).
3. Spin gradients at 25,000 RPM for 16 hours at 4 °C in SW28 rotor (In SW41 rotor, spin at 32,000 rpm for 15.5 hours).
4. Collect 70S peak using BRANDEL fractionator.
5. Remove sucrose by centrifugation at 4 °C in JA-17 rotor at 2800 RPM in CENTRICON 100 microconcentrators using 3-4 sequential 2 ml washes with buffer D. (Note: Ray used to soak the Centricons in 5% Tween-20 for 30 min, washed it with water then the appropriate buffer before using them.)
6. Immediately use the 70S for your experiments. Do not Freeze!

Buffers for In vitro Reconstitution of 30S Subunits & Subunit Association

Make buffers a day in advance and store them at 4 °C. Add 2-mercaptoethanol to the buffers just before use.

Buffer A:

20 mM NH_4^+ -Hepes (pH 7.5)
20 mM MgCl_2
500 mM NH_4Cl
0.01% Nikkol (CalBioChem)
4 mM 2-mercaptoethanol

Buffer B:

50 mM NH_4^+ -Hepes (pH 7.5)
15 mM MgCl_2
100 mM NH_4Cl
0.002% Nikkol

Buffer C:

20 mM NH_4^+ -Hepes (pH 7.5)
10 mM MgCl_2
100 mM NH_4Cl

Buffer D:

20 mM NH_4^+ -Hepes (pH 7.5)
20 mM MgCl_2
100 mM NH_4Cl