Arraystar Inc., 9430 Key West Ave #128, Rockville, MD 20850, USA
support@arraystar.com | 888-416-6343 | www.arraystar.com

Ribo-seq RNA Sample Preparation

(For customer use only)

Updated on 11/11/2026

Starting sample amount: 20 million cells
Typical RNA yield: > 3 ug RNA
RNA concentration: No requirement
RNA volume: No requirement
RNA integrity (e.g. RIN)**:** No requirement

A. Cell/Tissue Lysis

Prepare fresh buffers just before use.

- **Polysome Buffer 1** (1 mL): 20 mM Tris-HCl pH7.5, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 0.1 mg/mL cycloheximide (CHX), RNase-free ultrapure water to make up 1 mL total volume.
- **Lysis Buffer** (1 mL)**:** Polysome Buffer 1 supplemented with 1% Triton X-100, and 0.01 U/uL DNase I.

1. Aspirate culture media from one 10 cm dish of adherent cells. Place the dish on ice, gently wash with 5 mL ice-cold PBS, and aspirate the PBS thoroughly.
2. Drip 400 uL ice-cold Lysis buffer onto cells to cover the entire surface of the dish.
3. Scrape off the cells. Transfer the cells into a microcentrifuge tube on ice. Pipette the cells to disperse the cell clumps. Incubate on ice for 10 minutes.
4. Triturate cells ten times through a 26 gauge needle.
5. Clarify the lysate by 20,000 *g* centrifugation for 10 minutes at 4 °C. Recover the soluble supernatant.
6. The lysate can proceed to **RNase Digestion** or can be frozen at -80°C for storage/shipment.

B. RNase Digestion

1. To quantify the RNA content in the lysate, mix and vortex 2 uL aliquot of the lysate with 8 uL RNase-free ultrapure water. Measure the RNA concentration by a Qubit fluorometer (multiplied by the dilution factor of 5 to obtain the actual total RNA concentration).
2. Take the lysate volume containing 60 ug total RNA:

$$Lysate\ volume\ (uL) = \frac{60\ (ug)}{Lysate\ RNA\ concentration\ (ug/uL)}$$

Make up the final volume to 300 uL with Lysis Buffer.

3. Mix with 3 uL RNase I (Epicentre N6901K). Secure tight tube seal with Parafilm. Incubate at room

temperature for 45 minutes with rotary agitation.

4. Stop the reaction by adding and mixing with 10 uL SUPERase.In RNase inhibitor (Invitrogen AM2694, 20 U/uL).

C. Monosome Separation by Ultracentrifugation

Prepare fresh buffers just before use.

- **Sucrose Cushion Solution** (1 mL): 780 uL Polysome Buffer 1, 340 mg sucrose, and 5 uL SUPERase.In (Invitrogen AM2694, 20 U/uL).

1. Transfer the RNase I digested lysate to a 13x51 mm ultracentrifuge tube. Insert the pipette tip to the very bottom of the lysate phase and VERY slowly inject 0.9 mL Sucrose Cushion Solution. The lower sucrose will gently push the upper lysate phase upward. Do not disturb the phase separation.
2. Ensure the balancing centrifuge tube weighing exactly the same as the lysate centrifuge tube (< 15 mg difference).
3. Load the tubes to the ultracentrifuge rotor. Mark the tube outside with a marker pen to indicate the centrifugal sedimentation side. If using a Beckman Coulter Type 70 Ti rotor, spin at 50,800 RPM at 4 °C for 2 hours.
4. Remove all the supernatant. The ribosome sedimentation will appear as transparent or semitransparent glassy pellet.
5. Resuspend the pellet in 50 uL RNase-free 1X PBS. Transfer the solution to a centrifuge tube containing 450 uL TRIzol-LS. Mix well by shaking for 5 minutes.
6. Add 100 uL chloroform and vortex for 15 seconds. Incubate at room temperature for 5 minutes to facilitate phase separation prior to centrifugation. Centrifuge at 16,000 *g* at 4 °C for 15 minutes. Transfer 200 uL of the supernatant to a new tube.
7. Add 1 uL glycogen co-precipitant (e.g. GlycoBlue or Green Glycogen) and 20 uL 3M NaOAc (pH 5.2). Flicker to mix. Add 3X volume of absolute ethanol (prechilled at -20C). Keep at -80 °C for 30 minutes then centrifuge at 16,000 *g* at 4 °C for 30 minutes. Discard the supernatant. Wash the RNA pellet with 0.8 mL 80% ethanol (prechilled at -20 °C). Spin down and remove the residual ethanol with a micropipette (avoid touching the RNA pellet). Open the tube and air dry at room temperature for 8 minutes.
8. Dissolve the RNA in 15 uL RNase-free ultrapure water. Measure the RNA concentration with a Nanodrop instrument. Store the RNA sample at -80 °C.

The Ribo-seq RNA sample is now ready for shipment to Arraystar