Ribosome RNA Isolation kit Catalog: TBS6094-20

DESCRIPTION

Ribosome is short fragments (~28 nt) of the mRNA that it is translating in gene expression. It is also termed ribosome footprints or footprints. These short fragments of mRNA are protected from digestion with exogenous RNases in cell lysate. Therefore, these fragments can be isolated for sequencing and functional analysis.

Tribioscience's Ribosome RNA Isolation kit provides a streamlined approach to isolate ribosome RNA to meet life science research needs.

KIT CONTENTS (20 samples)

Part	Unit size	Storage
Polysome Buffer	5mL	-20℃
Lysis Buffer	12 mL	-20℃
10% Sucrose Buffer	30 mL	-20℃
50% Sucrose Buffer	30 mL	-20℃
Fast RNA Reagent	10 mL	4℃
RNase Solution	0.2mL	-20℃
RNA Carrier	0.1ml	-20℃
Nuclease-free Water	1 mL	-20℃

Kit do not provide reagents:

Isopropanol
Ethanol
Chloroform
RNase Inhibitor (20U/μL)

PROCEDURES

Ribosome Purification:

1. Add 0.4mL ice-cold Lysis Buffer into Frozen tissue, and incubate on ice for 10 min.
2. Homogenization: Use wide orifice tips to transfer tissue to a pre-chilled Dounce homogenizer. Tissues are slowly homogenized by hand (10-20 strokes).
3. Clarification: Homogenates are carefully transferred to clean 1.5 ml tubes with clean glass Pasteur pipets. Homogenates are clarified by centrifugation at 20,000g 4℃ for 10 min. Then collect the supernatants into a new tube (*Note: If you are not proceeding directly to nuclease footprint, flash freeze the lysate by immersion in liquid nitrogen, and store indefinitely at −80° C).*
4. Measure concentration: Measure the amounts of nucleic acid in the supernatants with Nanodrop (A260 units).
5. RNA Digestion: The homogenates are adjusted or diluted with lysis buffer to 0.3 ml for each gradient. Add 2ul RNase to 0.3mL samples, digest RNA at 37C for 15min.
6. Stop digestion reaction: Stop the digestion reactions by chilling on ice and adding 2.5μL RNase Inhibitor (Not provided in the kit) on ice for 10 min.
7. Clarification: Digested homogenates are clarified again by centrifugation at 20,000g 4℃ for 10 min. The supernatants are used to 10%-50% sucrose gradients.
8. Transfer the digestion to a 13 mm × 51 mm polycarbonate ultracentrifuge tube and underlay 0.9 ml sucrose cushion by carefully positioning a pipette tip at the very bottom of the tube and slowly

dispensing the sucrose solution. The lysate should float on top of the sucrose, leaving a visible interface between the layers.

9. Pellet ribosomes by centrifugation in a TLA100.3 rotor at 100,000 rpm, at 4 °C for 1 h, or 70,000 rpm for 2 h at °C
10. Mark the outside edge of the ultracentrifuge tube, where the ribosome pellet will be found, before removing the tube from the rotor. Gently pipette the supernatant out of the tube. The ribosomal pellet is glassy and translucent, and may not be visible until the supernatant is removed. The ribosomal pellet will be used for RNA purification.

Ribosome RNA isolation:

11. Resuspend the ribosomal pellet in 0.5 ml Fast RNA Reagent (*Note: The RNase free conditions from this step*).
12. Add 0.2ml chloroform and shake vigorously by hand or vertex for 10s. Then centrifuge for 10 min at 12,000g at 4°C.
13. Transfer carefully the upper aqueous phase (about 0.2 ml) to a clean tube.
14. Add 0.2ml (1 vol.) of isopropanol, 1µl RNA carrier, mix well and incubate the sample for 1 h at -20°C.
15. Centrifuge at 20,000g at 4°C for 30 min to precipitate the RNA, Discard the supernatant carefully.
16. Air dry for 5-10 min (Never let the RNA pellet air-dry completely).
17. Dissolve the RNA pellet in 20-50 µl of RNase-free water. RNA may be stored overnight at −20°C or for months at −80°C.

Research use only.