TOTAL ,SMALL, & MICRO RNA ISOLATION KIT RE-027& RE-027s

The miRNA Isolation Kit is designed for purification of microRNAs (miRNAs), small RNAs and total RNAs (from 18 to 200 nucleotides) from tissue samples, cultured cells, plasma, blood, serum, biological liquids and exosome complex.
Purification of miRNAs allows research into biological significant pathways for gene regulation.
The standard protocols for isolating total RNA and mRNA are not optimized for isolation of small RNA molecules and result in the loss of substantial amounts of miRNAs and other small RNAs. In addition, removal of the predominant larger RNAs is required for accurate analysis of miRNA expression by qPCR or microarray analysis.

The method employs a spin column with a silica-based fiber matrix that binds RNA in the presence of a chaotropic salt. The method is based on the selective binding of RNA molecules of different sizes to the silica-based fiber matrix when different ethanol concentrations are present in the solvent.

Sampling : Up to 100 mg tissue - 1x10^6 cultured cells - from 100 ul of Plasma

Handling Time: 30 minutes

Required Material

Ethanol, Chloroform
Phenol-ddH2O saturate
Isopropanol
Microcentrifuge
Water Bath or Dry Bath or Microwave Oven

Cat. No.	Product Name	Kit Contents	RE-027-50 50 RXN	RE-027S 5 RXN
RE-027	Total, Small, Mini & Micro RNA Isolation Kit	Lysis Buffer 2M NaOAcm pH 5.2 Wash Buffer (concentrated) Release Buffer RNA Column Collection Tube	12,5 mL 1,2 mL 7 mL** 3 mL 100 pcs 100 pcs	1,5 ml 150 ul 1 ml* 0,32 ml 2x5 pcs 2x5 pcs

Add *4 ml of RNase-free ethanol (96~100%) to Wash Buffer when first open.
Add **28 ml of RNase-free ethanol (96~100%) to Wash Buffer when first open.

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Important Notes

1. Make sure everything is RNase-free when handling RNA extraction.
2. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
3. Add 28 ml of RNase-free ethanol (96-100%) to Wash Buffer when first open for RE-027-50.

miRNA Protocol General Protocol :

Read the Important Notes before starting the following steps. HINT: Preheat Release Buffer to 65°C for step 15.

1. Add 200 µl Lysis Buffer into the tube containing up to 100 mg tissue or $1x10^6$ cultured cell pellet.
2. Vigorous mixing by vortexing. Incubate at room temperature for 10 minutes.
3. Add 20 µl 2M NaOAc, pH 5.2.
4. Add 180 µl ddH2O saturated phenol and 40 µl chloroform into the tube, vortex vigorously for 2 minutes.
5. Centrifuge at 12,000 rpm for 3 minutes. Transfer the upper phase into a clean tube.
6. Add ethanol to 35% volume (ex., add 108 µl ethanol to 200 µl upper phase). Mix well.
7. Transfer to the RNA Column in the Collection Tube. Incubate for 1 minute.
8. Centrifuge at 12,000 rpm for 30 seconds. Collect the filtrate.
9. Add ethanol to 70% volume (ex., add 338 µl ethanol to 290 µl upper phase). Mix well.
10. Transfer to another RNA Column in the Collection Tube. Incubate for 1 minute.
11. Centrifuge at 12,000 rpm for 30 seconds (miRNA bound to the column membrane).
12. Add 200µl Wash Buffer (ethanol added). Incubate for 1 minute.
13. Centrifuge at 12,000 rpm for 1 minute to completely remove the residue liquid.
14. Put the RNA Column to a clean 1.5 ml tube.
15. Add 50 µl Release Buffer (preheated to 65°C) to the center of column. Incubate for 3 minutes.
16. Centrifuge at 12,000 rpm for 3 minute to recover miRNAs along with small RNAs and tRNAs. (Note: The purified miRNAs can be further concentrated by standard ethanol precipitation procedure and then re-dissolved in a small volume ddH2O or TE, pH 8.0).
17. Use 1/5 volume to run on a mini agarose gel (or more accurately, a polyacrylamide gel) to check its quality. The majority of RNA visible on the gel should be <100 nt in size, with the major bands corresponding to total RNAs. The 5S and 5.8S rRNA species may also be visible. These total RNA and small rRNA bands should be clear and distinct. miRNA (21-22 nt) are typically not visible on the gel image.

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Important Notes

4. Make sure everything is RNase-free when handling RNA extraction.
5. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
6. Add 28 ml of RNase-free ethanol (96-100%) to Wash Buffer when first open to RE-027-50.

Protocol for Isolation of Total RNA (including micro & other Small RNAs)

Read the Important Notes before starting the following steps. **HINT: Preheat Release Buffer to 65°C for step 12.**

1. Add 200 µl Lysis Buffer into the tube containing up to 100 mg tissue or $1x10^6$ cultured cell pellet.
2. Vigorous mixing by vortexing. Incubate at room temperature for 10 minutes.
3. Add 20 µl 2M NaOAc, pH 5.2.
4. Add 180 µl ddH2O saturated phenol and 40 µl chloroform into the tube, vortex vigorously for 2 minutes.
5. Centrifuge at 12,000 rpm for 3 minutes. Transfer the upper phase into a clean tube.
6. Add ethanol to 70% volume (example: add 513 µl ethanol to 220 µl upper phase). Mix well.
7. Transfer to the RNA Column in the Collection Tube. Incubate for 1 minute.
8. Centrifuge at 12,000 rpm for 30 seconds. Collect the filtrate
9. Add 0µl Wash Buffer (ethanol added). Incubate for 1 minute.
10. Centrifuge at 12,000 rpm for 1 minute to completely remove the residue liquid.
11. Put the RNA Column to a clean 1.5 ml tube.
12. Add 50 µl Release Buffer (preheated to 65°C) to the center of column. Incubate for 3 minutes
13. Centrifuge at 12,000 rpm for 30 seconds to recover Total RNA.

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