*Note: for laboratory research use only*

# Universal Plant microRNA Extraction Kit (Spin-column)

**Cat. # RP5331 (50 preps)**

Signalway Biotechnology

## Ⅰ. Kit Content, Storage, and Stability

| **Content** | **Storage** | 50 preps (DP5331) |
|---|---|---|
| Buffer PL | RT<br>(-20℃ long term) | 15 ml |
| Proteinase K | -20℃ | 1 ml |
| Buffer MRL | 4℃in the dark | 55 ml |
| Buffer RW | 4℃ (one month)<br>-20℃ (long term) | 15 ml<br>***Add 60 ml ethanol before use.*** |
| RNase-free $H_2O$ | RT | 10 ml |
| Buffer RH | RT | 9 ml RNase-free $H_2O$<br>***Add 21 ml ethanol before use.*** |
| RNase-free Spin-column RA | RT | 50 |
| RNase-free Spin-column RB | RT | 50 |
| Collection Tube (2ml) | RT | 100 |

***All reagents are stable for 12 months if stored properly.***

## Reminder:

1. ***Add ethanol to Buffer RW and RH before use, mix adequately, and then check the box on the label showing ethanol was added!***
2. Precipitate may occur under low temperature, and it will affect RNA yield. Incubate at 37 °C **until buffer becomes clear**; let the buffer cool to RT before use. Buffer MRL can be transported at RT and should be stored at **4°C in the dark**.
3. Keep all of the reagents lids tightly caped when not in use to prevent evaporation, oxidation, and changes in pH.

## Ⅱ. Principle:

The kit applies improved guanidine thiocyanate-phenol one-step method to lyse samples and inactivate ribonucleases. Genomic DNA and 18s and 28s RNA are removed by the spin-column RA. Then microRNA (including miRNA, snRNA, and other RNA less than 200bp) is absorbed by the spin-column RB. After a serial of elution-centrifugation steps to remove cellular metabolite and proteins, microRNA will be eluted from silica membrane by using low salt RNase-free water.

## Ⅲ. Features:

1. Rapid and convenient. The kit does not contain poisonous phenol and does not need a step of ethanol precipitation.
2. Multi-elution can ensure high-purified microRNA, which is suitable for any types of molecular biology experiments.
3. High-purity; unique membrane absorption and specialized washing for removing protein and other debris.
4. Excellent microRNA yield.

## Ⅳ. Notes

***Please read this section before your experiment.***

1. **To prevent RNA degradation, all the centrifugation steps should be performed under 4°C unless being specially noted**. Recommend using traditional centrifuge (with up to 13,000 rpm), such as Eppendorf 5415C.
2. Reagent and buffer may contain a corrosive compound; wear latex gloves to avoid direct contact with skin, eyes, and clothes. If contact occurs, wash with water or physiological saline.
3. Due to the prevalence of RNases, wear gloves during the procedure and change them whenever contact by reagents occurs; please follow standard laboratory procedures of "Molecular Clone" rules.

** Wear gloves during entire process. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases.*

** Use sterile, disposable plastic-ware, and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any non-disposable items (such as automatic pipettes) can be rich sources of RNases.*

** Treat non-disposable glassware and non-disposable plastic-ware before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plastic-ware with 0.1N NaOH with 1mM EDTA followed by RNase-free water.*

4. The integrity of purified RNA may be determined by denaturing agarose gel electrophoresis (or agarose gel electrophoresis). The ratio of 28S to 18S ribosomal RNA should be approximately 2:1 by ethidium bromide staining. There may be the third band about 0.1-0.3 kb (5S RNA and tRNA) and even fourth or fifth band of RNA purified from some plant tissues. The extracted preRNA, hnRNA, and small RNA will appear as some bands at sizes of 7-15 kb.
5. Chloroform and isoamylol should be prepared by the user.
6. The routine method to determine the yield and purity of RNA is spectrophotometry ($OD_{260}/OD_{280}$). Please dissolute RNA by TE. Water will make $OD_{280}$ higher because of lower ion intensity and PH.
7. The sample, mixed with Buffer MRL and homogenized (without chloroform), can be stored under –60℃ to –70℃ for a month even longer.

## Ⅴ.Procedure

***Please add proper volume of ethanol to buffer RW and RH before use.***

1. Weigh approximate 100 mg tissues and grind with liquid nitrogen.

   Optional: Homogenize sample in 10 volumes buffer PL (e.g., 1 ml buffer PL per 50-100 mg tissues) when liquid nitrogen is not used.

2. Transfer the sample to an RNase-free 1.5 ml centrifuge tube, add 1 ml Buffer PL, mix well, and incubate at 65℃ for 5 min (overturn several times to mix thoroughly to degrade the nucleoprotein thoroughly.

3. Centrifuge at 12,000 rpm for 5 min at RT. Pipette the supernatant to an RNase-free centrifuge tube.

4. Add equal volume of pre-cooled **isoamylol, overturn to mix thoroughly, then centrifuge at 12,000 rpm for 5 min at 4** ℃.

5. Remove supernatant (do not discard the pellet), add 100 μl RNase-free $H_2O$ to dissolve the pellet.

6. Add 900 μl Buffer MRL, vortex and mix vigorously, and keep at RT for 2 min

7. Add 200 μl chloroform, vortex and mix vigorously, and then incubate for 2-3 min at RT.

8. Centrifuge mixture at 12,000 rpm for 10 min at 4°C. Remove upper, aqueous phase that RNA is present to a clean, sterile, and DEPC-treated eppendorf tube.

9. Precipitate the aqueous phase by addition of a 0.6 volume of Buffer RH, mix gently. Flocculated precipitate may appear.

10. Put solution from step 9 and the flocculated precipitate into a Spin-column RA (place the spin-column to collection tube).

11. Centrifuge at 10,000 rpm for 45 seconds, and collect flow-through (including micro RNA).Check the volume of flow-through and add Buffer RH (2/3 volume of flow-through) and mix gently, then put this mixed solution into a Spin-column RB, centrifuge at 10,000g for 30 seconds at 4°C (Repeat the step until all solution is loaded), and discard flow-through. At this stage, you have Spin-column RA containing macro RNA (18s and 28s) and Spin-column RB containing microRNA.

    **If you want to harvest macro RNA (18s and 28s) from Spin-column RA, please proceed Step 12-16 with the spin column RA.**

    **If you want to harvest microRNA from Spin-column RB, please proceed Step 12-16 with the spin column RB.**

12. Add 700 µl buffer RW (**please check if ethanol is added**), centrifuge at 12,000 rpm for 60 seconds, and discard flow-through.
13. Add 500 µl buffer RW, centrifuge at 12,000 rpm for 60 seconds, and discard flow-through.
14. Place spin-column RB back to collection tube, centrifuge at 12,000 rpm for 2 min, and discard supernatant to avoid ethanol inhibition of the down-stream procedures.
15. Place the Spin-column into a new RNase-free tube, add 60-80 µl RNase-free H2O (Pre-warmed at 65-70℃), hold for 2 min at RT. Centrifuge at 12,000 rpm for 1 min.
16. Keep microRNA at -20℃ or apply for down-stream reactions.

# Ⅶ.Troubleshooting

| Problem | Possible Reason | Advices |
|---|---|---|
| Low microRNA | Tissue not homogenized thoroughly | For tissue in liquid nitrogen, grind tissue into fine powder; after adding buffer RL, completely lyse cells by pipetting or vortexing; for fresh tissue or plant tissue, grind tissue in Buffer MRL using a mortar and pestle. |
| | RNA degraded | Using fresh samples |
| | low RNA in samples | The amount of RNA varies in tissues and cells. Some “low RNA” samples need larger amount of tissues or cells for homogenization. |
| | Beyond the binding capacity of silica membrane | Using multiple spin-columns RA for the same sample. |
| | Ethanol not added to Buffer RW | ***Add the ethanol before use.*** |
| $OD_{260}$/ $OD_{280}$ <1.6 | Dissolute RNA by water, which will make $OD_{280}$ higher because of lower ion intensity and pH. | Dissolute RNA by TE for spectrophotometry analysis. |
| | Contaminated by proteins and phenol | Don’t take middle or lower phases in Step 8 and ensure proceeding of Step 11. |
| Genomic DNA contamination | Initial sample beyond the capacity of Buffer MRL | Selecting appropriate amount of sample. |
| | Sample contains some chemical solvent (such as ethanol, DMSO etc). | Avoid contamination of sample |
| | Extract middle phases in Step 5. | Don’t take middle phases in Step 8. |
| Degradation and poor integrity of RNA | Non-disposable glassware and plasticware not treated before use. | Treat non-disposable glassware and plasticware before use to ensure that it is RNase-free |
| | Samples were not properly prepared or stored. RNA may have been degraded during sample preparation. | To avoid RNA degradation within samples, immediately homogenize sample with Buffer MRL or freeze samples immediately in liquid nitrogen and store at -70°C if they cannot be immediately processed. |
| | | |
| | RNA not stored under –60℃ to –70℃ | Store under –60℃ to –70℃ |
| | RNA degradation in process. | It is essential to work quickly during sample preparation. Maintain sample lysate at 4°C during preparation. |

| | | |
|---|---|---|
| Down-stream RT-PCR not successful. | Forget to do step14, or when take the spin-column out, touching some flow-through including ethanol carelessly. Ethanol will inhibit RT-PCR. | Ensuring to do step14, take the spin-column out carefully, and then put outside for a few minutes for ethanol evaporation. |