# miRcute Plant miRNA Isolation Kit

High-efficiency miRNA extraction kit specially targeting plant samples

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# miRcute Plant miRNA Isolation Kit

**(Spin Column)**

**Cat. no. GDP504**

## Kit Contents

| Contents | GDP504 (50 preps) |
|---|---|
| Buffer SLM | 30 ml |
| Buffer PLA | 3 ml |
| Buffer RWA | 12 ml |
| Buffer MRD | 24 ml |
| RNase-Free dd$H_2O$ | 15 ml |
| RNase-Free Columns CS set | 50 |
| RNase-Free Columns miRspin set | 50 |
| RNase-Free Columns CR4 set | 50 |
| RNase-Free Centrifuge Tubes (1.5 ml) | 50 |
| Handbook | 1 |

## Storage

Buffer SLM added with β-mercaptoethanol can be stored at 2-8°C for one month. Other reagents should be stored at room temperature (15-30°C) for 15 months.

## Introduction

This kit can rapidly extract total RNA containing miRNA from plant tissues, especially plant tissues rich in polysaccharide and polyphenol or starch (such as cotton leaves, potato tubers, apples, peach tree leaves, etc.), and can simultaneously process a large number of different samples. The extracted total RNA has high purity and is free from protein and other impurities contamination. It can be used in various downstream experiments such as RT-PCR, Real Time RT-PCR, chip analysis, Northern Blot, Dot Blot, PolyA screening, *in vitro* translation, RNase protection analysis, molecular cloning etc.

## To avoid RNase contamination, please note that:

1. Change new gloves frequently, because the skin contains bacteria and may cause RNase contamination.
2. Use RNase-free plastic products and pipette tips to avoid cross contamination.
3. RNA will not be degraded by RNase in Buffer SLM. However, plastic and glassware without RNase should be used in the subsequent processing.
4. RNase-Free dd$H_2O$ should be used for the preparation of solutions (add water into a clean glass bottle, add DEPC to a final concentration of 0.1%(v/v), mix well, place overnight, and autoclave).

## Precautions Please read these precautions before use

1. Before operation, add β-mercaptoethanol to Buffer SLM to a final concentration of 5%. For example, add 25 μl of β-mercaptoethanol to 475 μl of Buffer. It's better to prepare the reagent when using. The prepared reagent can be stored for one month at 2-8°C.
2. Add 96-100% ethanol to Buffer MRD and Buffer RWA before the first use. Please refer to the label on the bottle for the added volume.
3. Carry out all extraction operations at room temperature.

## Protocol

Add 96-100% ethanol to Buffer RWA and Buffer MRD before using for the first time according to the label on the bottles.

**I. Extraction of miRNA enriched fractions**

If high purity miRNA is needed for the downstream applications, such as miRNA chip research and miRNA cloning, this protocol is recommended.

1. Homogenization

   Grind 50 mg of plant leaves or fruit pulp rapidly into powder in liquid nitrogen, transfer into a 1.5 ml centrifuge tube, add with 500 µl of Buffer SLM (please check whether β-mercaptoethanol has been added before use), mix evenly upside down to ensure that the buffer immerses all samples, then add with 1/10 volume of Buffer PLA, and mix evenly by vortex immediately.

   **Note 1: For plant samples with an expected RNA yield of less than 10 µg, please use a starting sample size of 100 mg. For starch-rich samples or mature leaves, increase the amount of Buffer SLM to 700 µl.**

   **Note 2: Due to the rich diversity of plants and the variable RNA content of the same plant at different growth stages and in different tissues. please select the appropriate sample size according to the specific experimental conditions.**

2. Centrifuge at room temperature at 12,000 rpm (~ 13,400×g) for 5 min.
3. Transfer the supernatant to the RNase-Free Filter Column CS (place the Filter Column CS in the collection tube), centrifuge at room temperature at 12,000 rpm(~13,400×g) for 2 min, carefully remove the supernatant from the collection tube into a new RNase-Free Centrifuge Tube, and avoid the tip from contacting the cell debris precipitation in the collection tube as much as possible.
4. Measure the volume of transfer liquid, slowly add 96-100% ethanol with 0.43 times the volume of transfer liquid (e.g. add 215 µl 96-100% ethanol to 500 µl transfer liquid ), and mix evenly (precipitation may occur at this time). Transfer the obtained solution and the precipitate into an RNase-Free Column miRspin, and centrifuge at room temperature of 12,000 rpm (~ 13,400×g) for 30 sec. If the solution cannot be added to the RNase-Free Columns miRspin at one time, please transfer them in two times, discard the Column miRspin after centrifugation, and retain the flow through.
5. Measure the volume of flow through, slowly add 0.75 times of 96-

100% ethanol (e.g. 700 µl flow through plus 525 µl 96-100% ethanol) into the flow through, and mix evenly (precipitation may occur at this time). Transfer the obtained solution and precipitate into a RNase-Free Column CR4 together, and centrifuge at room temperature at 12,000 rpm (~13,400×g) for 30 sec. If the solution cannot be added into RNase-Free Column CR4 at one time, please transfer in two times, discard the flow through after centrifugation, and retain Column CR4.

6. Add 700 µl of Buffer MRD **(ensure that ethanol has been added before use)** to Column CR4, and let the mixture to stand at room temperature for 2 min. Centrifuge at room temperature at 12,000 rpm (~13,400×g) for 30 sec, and discard the flow through.
7. Add 500 µl of Buffer RWA **(ensure that ethanol has been added before use)** to Column CR4, and let it stand at room temperature for 2 min. Centrifuge at room temperature at 12,000 rpm (~13,400×g) for 30 sec, and discard flow through.
8. Repeat step 7.
9. Place the Column CR4 in a 2 ml collection tube and centrifuge at 12,000 rpm (~ 13,400×g) for 2 min at room temperature to remove residual liquid.

   **Note: The purpose of this step is to remove the residual Buffer RWA in the column. After centrifugation, place the Column CR4 at room temperature for 2 min, or place it on an ultra-clean bench for ventilation for a moment to fully dry. If there is buffer residue, it may affect subsequent RT and other experimental operations.**
10. Transfer Column CR4 into a new RNase-Free 1.5 ml centrifuge tube, add 50 µl RNase-Free dd$H_2O$, leave at room temperature for 2 min, and centrifuge at room temperature at 12,000 rpm (~13,400×g) for 2 min.

    **Note: The volume of elution buffer should not be less than 30 µl. Too small volume will affect the recovery efficiency. And RNA should be kept at -70**°C **to prevent degradation. To improve RNA yield, repeat the previous step once.**

## II. Extraction of Total RNA

The extracted total RNA contains small RNA such as miRNA. This method can be used when the purity requirement of miRNA is not high, for example miRNA RT-PCR and miRNA Northern blot.

1. Homogenation

Grind 50 mg of plant leaves or fruit pulp rapidly into powder in liquid

nitrogen, transfer into a 1.5 ml centrifuge tube, add with 500μl of Buffer SLM **(ensure β-mercaptoethanol has been added before use)**, mix upside down evenly to ensure that the buffer immerses all samples, then add buffer PLA with 1/10 the volume of Buffer SLM, and mix evenly by vortex immediately.

**Note 1: For plant samples with an expected RNA yield of less than 10 μg, please use a starting sample size of 100 mg. For starch-rich samples or mature leaves, increase the amount of Buffer SLM to 700 μl.**

**Note 2: Due to the rich diversity of plants and the variable RNA content of the same plant at different growth stages and in different tissues. please select the appropriate sample size according to the specific experimental conditions.**

2. Centrifuge at room temperature at 12,000 rpm (~13,400×g) for 5 min.
3. Transfer the supernatant to the Filter Column CS (place the Filter Column CS in the collection tube), centrifuge at 12,000 rpm (~13,400×g) for 2 min, carefully remove the supernatant from the collection tube into a new RNase-Free centrifuge tube, and avoid the tip from contacting the cell debris precipitation in the collection tube as much as possible.
4. Slowly add 1.5 times the volume of 96-100% ethanol in the supernatant, mix well (precipitation may occur at this time), transfer the obtained solution and precipitation together into the Column CR4, centrifuge at 12,000 rpm (~13,400xg) for 30 sec, discard the flow through in the collection tube, and put the Column CR4 back into the collection tube. If all the solution cannot be transferred to Column CR4 at one time, please proceed in two steps.

   **Note: If there's loss in the volume of supernatant, please adjust the dosage of ethanol accordingly.**
5. Add 700 μl of Buffer MRD **(ensure that ethanol has been added before use)** to Column CR4, centrifuge at 12,000 rpm (~13,400 x g) for 30 sec, discard the flow through in the collection tube, and return Column CR4 to the collection tube.
6. Add 500 μl of Buffer RWA **(ensure that ethanol has been added before use)** to Column CR4, centrifuge at 12,000 rpm (~13,400 x g) for 30 sec, discard the flow through in the collection tube, and return Column CR4 to the collection tube.
7. Repeat step 6.
8. Place the Column CR4 in a 2 ml collection tube and centrifuge at 12,000 rpm (~ 13,400 x g) for 2 min at room temperature to remove residual liquid.

**Note: The purpose of this step is to remove the residual Buffer RWA in the column. After centrifugation, place the Column CR4 at room temperature for 2 min, or place it on an ultra-clean bench for ventilation for a moment to fully dry. If there is buffer residue, it may affect subsequent RT and other experimental operations.**

9. Transfer the Column CR4 into a new RNase-Free 1.5 ml centrifuge tube, add 50 µl RNase-Free dd$H_2O$, leave at room temperature for 2 min, and centrifuge at room temperature of 12,000 rpm (~13,400×g) for 2 min.

   **Note: The volume of elution buffer should not be less than 30 µl. Too small volume will affect the recovery efficiency. And RNA should be kept at -70°C to prevent degradation. To improve the RNA yield, repeat the previous step once.**

## Determination of RNA Purity and Concentration

**Integrity:** The RNA integrety can be detected by common agarose gel electrophoresis (electrophoresis conditions: gel concentration 1.2%; 0.5×TBE electrophoresis buffer; 150 V, 15 min). Since 70-80% of RNA in cells is rRNA, very obvious rRNA bands should be visible under UV after electrophoresis with the sizes about 5 kb and 2 kb, which is equivalent to 28S and 18S rRNA respectively. Due to the large amount of chloroplast RNA in plant leaves, four or more rRNA bands can be seen. The brightest rRNA band in the plant RNA sample should be 1.5-2.0 times brighter of the second brightest rRNA band, otherwise it indicates degradation of the RNA sample. The appearance of smear or disappearance of bands indicates serious degradation of the sample.

**Purity:** $OD_{260}/OD_{280}$ ratio is an indicator to measure the degree of protein contamination. For high quality RNA, $OD_{260}/OD_{280}$ value is between 1.8 and 2.1, and a ratio of 2.0 is a marker of high quality RNA. $OD_{260}/OD_{280}$ value is affected by the pH of the solution used for the determination. For the same RNA sample, assuming that the $OD_{260}/OD_{280}$ value measured in 10 mM Tris and pH7.5 solution is between 1.8 and 2.1, the value measured in aqueous solution may be between 1.5 and 1.9, but this does not mean that RNA is impure.

**Concentration:** Take a certain amount of purified RNA, dilute it *n* times with RNase-Free dd$H_2O$, blank the spectrophotometer with RNase-Free dd$H_2O$, then measure the $OD_{260}$ and $OD_{280}$ value of the diluent, and calculate the RNA concentration according to the following formula:

Final concentration (ng/µl)= ($OD_{260}$)× (dilution factor *n*)×40