SECTION 1. PURIFICATION OF SMALL RNA FROM PLANT CELLS AND TISSUE

1.1 Sample Homogenization

- Both fresh and frozen plant tissues can be used for this protocol.

1.1.1 Sample Homogenization and Lysis

1. Transfer ≤100 mg of plant tissue or a maximum of 5 x 10^7 plant cells into a mortar that contains enough liquid nitrogen to cover the sample. Grind sample into a fine powder using a pestle in liquid nitrogen.
   Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen.
2. Allow the liquid nitrogen to evaporate without allowing the tissue to thaw.
3. Transfer the powder to a 1.5ml microcentrifuge tube (user supplied)
4. Add 600µl of Lysis Buffer RPX and vortex vigorously for 30s.
5. Assemble an ISOLATE II **BioFilter** with a supplied Collection Tube.
6. Pipette lysate onto the BioFilter and centrifuge for 2 min at 14,000 x g.
7. Pipette only the clear supernatant from the flow-through into an RNase-free 1.5ml microcentrifuge tube (user supplied). Note the volume of the supernatant/lysate.
   Note: Ensure only the clear supernatant is transferred, avoiding any of the debris at the bottom of the Collection Tube.
8. Add a volume of 96-100% ethanol that is a half of the lysate volume (e.g. 50µl of ethanol is added to every 100µl of lysate). Vortex to mix.
   Proceed to section 1.2.

1.2 Large RNA Removal

1. Assemble an ISOLATE II **Large RNA Removal Column** (white ring) with the supplied Collection Tube.
2. Apply the ethanolic lysate onto the column (from section 1.1.1, step 8) and centrifuge for 1 min at 14,000 x g. Transfer the flow-through, which contains the small RNA into an RNase-free 1.5ml microcentrifuge tube (user supplied).
   Important note: *The flow-through contains the small RNA, therefore ensure this fraction is not discarded.*
3. If the large RNA is to be isolated, retain the column and proceed to the **Optional Large RNA Purification Protocol (see section 2)**. Otherwise, discard the column.
   Important note: *The Large RNA Removal Column can be kept at 4°C for several hours or ≤15 min at room temperature. Freezing and thawing is not recommended.*

1.3 **Small RNA Capture**

1. Add 1 volume of 96-100% ethanol to the small RNA containing flow-through collected in section 1.2, step 2. For example, add 100µl of ethanol to every 100µl of collected flow-through. Vortex for 10s to mix.
2. Assemble an ISOLATE II **miRNA Column** (black ring) with a provided Collection Tube.
3. Apply half of the ethanolic lysate onto the column and centrifuge for 1 min at 14,000 x g.
4. Discard the flow-through and reassemble the spin column with the Collection Tube.
5. Repeat steps 3 and 4 to complete the capture of the small RNA.
   Optional: The ISOLATE II Plant miRNA Kit purifies small RNA with minimal amounts of genomic DNA contamination. However, for sensitive applications, an optional on-column DNA removal protocol is provided (see full manual Appendix A). DNase I treatment should be performed at this point in the protocol with the supplied DNase I Solution and DNase I Reaction Buffer DRB.

1.4 **miRNA Column Wash**

1. Apply 400µl of Wash Buffer W1 to the ISOLATE II **miRNA Column** (black ring) and centrifuge for 1 min at 14,000 x g.
   Note: Ensure the entire wash buffer volume has passed into the Collection Tube by inspecting the column. If the entire wash volume has not passed through, centrifuge for an additional 1 min at 14,000 x g.
2. Discard the flow-through and reassemble the spin column with the Collection Tube.
3. Repeat steps 1 and 2 to wash column a second time.
4. Wash column a third time by adding 400µl of Wash Buffer W1 and centrifuge for 1 min at 14,000 x g.
5. Discard flow-through and reassemble spin column with its Collection Tube.
6. Centrifuge for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the Collection Tube.

1.5 **Small RNA Elution**

1. Place the ISOLATE II **miRNA Column** into a fresh 1.7ml Elution Tube (supplied).
2. Add 50µl of RNA Elution Buffer to the column.

   Note: For more concentrated RNA, use a lower volume of RNA Elution Buffer (a minimum of 20µl is recommended).
3. Centrifuge for 2 min at 200 x g, followed by 1 min at 14,000 x g. Note the volume eluted from the column. If the entire volume has not been eluted, spin column for an additional 1 min at 14,000 x g to elute the RNA.

   Note: For maximum RNA recovery, it is recommended to apply a second volume of 20-50µl RNA Elution Buffer and elute into the same microcentrifuge tube (repeat steps 2 and 3). Alternatively, re-apply the first eluate onto the column and re-elute into the same microcentrifuge tube (for higher concentration).

1.6 **Storage of RNA**

The isolated RNA can be stored at -20°C for up to three days or at -80°C (recommended) for long term storage.

SECTION 2. OPTIONAL LARGE RNA PURIFICATION PROTOCOL

2.1 **Large RNA Column Wash**

1a. Reassemble the **ISOLATE II Large RNA Removal Column** (white ring) with the Collection Tube used in section 1.2 step 2.

Optional: The ISOLATE II Plant miRNA Kit purifies large RNA with minimal amounts of genomic DNA contamination. However, for sensitive applications, an optional on-column DNA removal protocol is provided (see full manual Appendix A). DNase I treatment should be performed at this point in the protocol with the supplied DNase I and Reaction Buffer DRB.

1b. Apply 400µl of Wash Buffer W1 to the spin column and centrifuge for 1 min at 14,000 x g.

Note: Ensure the entire wash buffer volume has passed into the Collection Tube by inspecting the column. If the entire wash volume has not passed through, centrifuge for an additional 1 min at 14,000 x g.

2. Discard the flow-through and reassemble the spin column with the Collection Tube.
3. Repeat steps 1b and 2 to wash the column a second time.
4. Wash the column for a third time by adding 400µl of Wash Buffer W1 and centrifuge for 1 min at 14,000 x g.
5. Discard the flow-through and reassemble the spin column with its Collection Tube.
6. Centrifuge the column for 2 min in order to thoroughly dry the column. Discard the Collection Tube.

2.2 Large RNA Elution

1. Place the ISOLATE II **Large RNA Removal Column** (white ring) into a fresh 1.7ml Elution Tube (supplied).
2. Add 50µl of RNA Elution Buffer to the column.
3. Centrifuge for 2 min at 200 x g, followed by 1 min at 14,000 x g. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column for an additional minute at 14,000 x g to elute the RNA.

   Note: For maximum RNA recovery, it is recommended to apply a second volume of 50µl RNA Elution Buffer and elute into the same microcentrifuge tube (repeat steps 2 and 3). Alternatively, re-apply the first eluate onto the column and re-elute into the same microcentrifuge tube (for higher concentration).

2.3 Storage of RNA

The isolated RNA can be stored at -20°C for up to three days or at 80°C (recommended) for long term storage.

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Plant miRNA Kit (Phenol free)	25 Preps	BIO-52084

Technical Support
The troubleshooting guide is provided in the online protocol.
For technical support please email us at tech@bioline.com
or visit www.bioline.com