

SECTION 1. LYSATE PROTOCOLS

- 1 To prepare lysate from your starting material, please refer to the supplied manual or download the online protocol (www.bioline.com/isolate).

SECTION 2. PURIFICATION OF SMALL RNA FROM ALL TYPES OF LYSATE

2.1 Large RNA Removal

1. Assemble an ISOLATE II **Large RNA Removal Column** (white ring) with the provided Collection Tube.
2. Apply the ethanolic lysate onto the column (from section 1) and centrifuge for 1 min at 14,000 x g. Transfer the flow-through, which contains the small RNA species into a RNase-free 1.5ml microcentrifuge tube (user supplied).
Important note: *The flow-through contains the small RNA, therefore ensure that 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 (section 3)**. 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.*

2.2 Small RNA Capture

1. **For eukaryotic cells, blood, biofluid or bacteria:** Add 350µl of 96-100% ethanol to the small RNA containing flow-through collected in step 3. Vortex for 10s to mix.
For mammalian tissue: Add 1 volume of 96-100% ethanol to the small RNA containing flow-through collected in step 3. 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 the provided Collection Tube.
3. Apply half of the ethanolic lysate onto the column and centrifuge for 1 min at $\geq 3,500 \times g$.
Note: Ensure the entire lysate volume has passed into the Collection Tube by inspecting the column. If the entire lysate volume has not passed through, centrifuge for an additional 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.

Technical Support

The troubleshooting guide is provided in the online protocol.
For technical support please email us at tech@bioline.com
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6. **Optional:** The ISOLATE II 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 and Reaction Buffer DRB.

2.3 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 the column a second time.
4. Wash the column 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 for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the Collection Tube.

2.4 Small RNA Elution

1. Place the ISOLATE II **miRNA Column** (black ring) 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). See section 7.3.4 of the full manual for further details on alternative elution procedures.

2.5 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.

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SECTION 3. OPTIONAL LARGE RNA PURIFICATION PROTOCOL

3.1 Large RNA Column Wash

- 1a. Reassemble the ISOLATE II **Large RNA Removal Column** (white ring) with the Collection Tube used in section 2.1 step 2.

Optional: The ISOLATE II 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 that 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 dry the column thoroughly. Discard the Collection Tube.

3.2 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). See section 7.3.4 of the full manual for further details on alternative elution procedures.

3.3 Storage of RNA

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

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II miRNA Kit (Phenol free)	50 Preps	BIO-52083

BTP0115V1

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