Presto™ miRNA Purification Kit Protocol

For research use only

Catalogue Number
PMI004, PMI050, PMI100

Introduction

The Presto™ miRNA Purification Kit is designed for purifying and enriching of small RNA molecules (< 200 nt) from a wide variety of samples, such as animal tissues, cultured cells and bacterial cells. These small RNAs include microRNA (miRNA), short interfering RNA (siRNA), as well as tRNA and 5S ribosomal RNA. miRNAs and siRNAs are typically 20-25 nucleotides long and vital for regulating gene expression by binding to mRNA molecules and affecting their stability or translation. Purification is based on spin column technology without the use of phenol and chloroform. Briefly, the cells or tissues are lysed using detergents and chaotropic salt. The larger RNA fragments in the chaotropic salt are bound by the glass fiber matrix of the first spin column while the smaller RNA molecules pass through into the flow-through. The smaller RNA molecules in the flow-through are bound by the second spin column once any contaminants have been removed using the Wash Buffer. The small RNA molecules are then eluted using the RNase-free water, and are ready to be used in various applications, including RT-PCR, northern blotting and microarray analysis.

Components

	PMI004	PMI050	PMI100
PMI Buffer	2 ml	30 ml	60 ml
W1 Buffer	2 ml	30 ml	50 ml
Wash Buffer[1] (Add ethanol)	2 ml (8 ml)	25 ml (100 ml)	50 ml (200 ml)
RNase-free water	1 ml	6 ml	15 ml
RB column	8 pcs	50 pcs x2	50 pcs x4
2 ml Collection Tube	8 pcs	50 pcs x2	50 pcs x4

[1]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Caution

PMI Buffer contains chaotropic salt. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Steps to prevent RNase contamination

- Disposable gloves should be worn at all times during RNA extraction procedure, changing gloves frequently to avoid contamination is recommended.
- Disposable and nondisposable plasticware and automatic pipettes should be sterile and used only for RNA procedures.
- Clean the bench and all equipment surfaces with commercially available RNase decontamination solutions.

Additional Requirements

absolute ethanol, ß-mercaptoethanol, microcentrifuge tubes, pipette tips.

- For cell samples: phosphate-buffered saline (PBS), 0.10-0.25% trypsin.
- For tissue samples: liquid nitrogen, 20-G needle syringe, mortar and pestle.
- For bacteria samples: lysozyme, bacteria lysis buffer. (lysozyme and bacteria lysis buffer can be purchased from Geneaid)

1. Sample Homogenization and Lysis

Sample	Procedure
Adherent Cultured Cells	● Remove the culture medium and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS from the culture dish. ● Directly add **300 µl of PMI Buffer** and **3 µl of ß-mercaptoethanol** to the culture dish. ● Lyse the cells (up to 3 x 10^6 cells) directly in the culture dish by gently tapping culture dish and swirling buffer around plate surface for 5 minutes. ● Transfer the sample to a 1.5 ml microcentrifuge tube (RNase-free). ● Add **120 µl of absolute ethanol** to the sample lysate. Mix by vortex for 10 seconds. ● Proceed to step 2.
Suspension Cultured Cells	● Transfer cells (up to 3 x 10^6) to a 1.5 ml microcentrifuge tube (RNase-free). ● Harvest by centrifugation at 300 x g for 5 minutes then remove the culture medium completely. ● Add **300 µl of PMI Buffer** and **3 µl of ß-mercaptoethanol** to the cell pellet then mixed several times by pipette. Ensure that the entire pellet is completely dissolved. ● Incubate the sample mixture for 5 minutes at room temperature. ● Add **120 µl of absolute ethanol** to the sample lysate. Mix by vortex for 10 seconds. ● Proceed to step 2.
Animal tissue	● Cut off 10-15 mg of fresh/frozen tissue, freeze tissue in liquid nitrogen then grind thoroughly with a mortar and pestle. Transfer the tissue powder to a 1.5 ml microcentrifuge tube (do not allow the tissue to thaw). NOTE: If using frozen animal tissue, the sample must have been flash frozen in liquid nitrogen and immediately stored at -70ºC until use to avoid RNA degradation. ● Add **400 µl of PMI Buffer** and **4 µl of ß-mercaptoethanol** to the 1.5 ml microcentrifuge tube. ● Shear the tissue by passing lysate through a 20-G needle syringe 10 times then incubate at room temperature for 5 minutes. ● Centrifuge at 12-16,000 x g for 2 minutes, transfer 300 µl of supernatant to a clean 1.5 ml microcentrifuge tube (RNase-free). ● Add **120 µl of absolute ethanol** to the sample lysate. Mix by vortex for 10 seconds. ● Proceed to step 2.
Bacteria	● Transfer bacteria cells (up to 1 x 10^9) to a 1.5 ml microcentrifuge tube (RNase-free). ● Centrifuge at 12-16,000 x g for 2 minutes then remove the supernatant completely. ● Weigh and transfer 10 mg of lysozyme powder to a new 1.5 ml microcentrifuge tube (RNase-free). ● Add 1 ml of bacteria lysis buffer to the microcentrifuge tube containing 10 mg of lysozyme. Vortex the tube until the lysozyme powder is completely dissolved. ● Add **100 µl of bacteria lysis buffer containing lysozyme** to the bacteria cell pellet. ● Resuspend the cell pellet by vortex or pipetting. NOTE: Residual bacteria lysis buffer containing lysozyme should be stored at 4ºC for 2 weeks. ● Incubate the sample for 5 minutes at room temperature. ● Add **200 µl of PMI Buffer** and **2 µl of ß-mercaptoethanol** to the sample lysate, mix well by vortex then incubate at room temperature for 5 minutes. ● Add **120 µl of absolute ethanol** to the sample lysate. Mix by vortex for 10 seconds. ● Proceed to step 2.
Biological fluids	● Transfer 100 ul of liquid sample to a microcentrifuge tube. ● Add **300 µl of PMI Buffer** and **3 µl of ß-mercaptoethanol** to the sample then mix by vortex for 15 seconds. ● Incubate the sample mixture at room temperature for 5 minutes. ● Centrifuge the sample at 12-16,000 x g for 1 minute to remove cell debris then transfer 300 µl of the clear supernatant to a new microcentrifuge tube. ● Add **120 µl of absolute ethanol** to the sample lysate. Mix by vortex for 10 seconds. ● Proceed to step 2.

2. Large RNA Removal

- Place a **RB Column** in a 2 ml Collection Tube and transfer all of the sample mixture to the RB Column.
- Centrifuge at 14-16,000 x g for 1 minute.
- Transfer the filtrate which contains the small RNA molecules to a new 1.5 ml microcentrifuge tube (RNase-free).

NOTE: If large RNA fragments are to be purified, retain the column and proceed to the Optional Large RNA Purification Protocol (Appendix A). Otherwise, discard the column.

3. Small RNA Enrichment

- Add **280 µl of absolute ethanol** to the filtrate. Mix by vortex for 10 seconds.
- Place a **new RB Column** in a new 2 ml Collection Tube and transfer all of the sample mixture to the RB Column.
- Centrifuge at 14-16,000 x g for 1 minute to allow the small RNA molecules to bind to the second RB Column membrane.
- Discard the flow-through then place the RB Column back in the 2 ml Collection Tube.

4. RNA Wash

- Add **600 µl of Wash Buffer (make sure ethanol was added)** into the RB Column.
- Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.
- Place the RB Column back in the 2 ml Collection Tube.
- Add **600 µl of Wash Buffer (make sure ethanol was added)** into the RB Column.
- Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.
- Place the RB Column back in the 2 ml Collection Tube and centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

5. Small RNA Elution

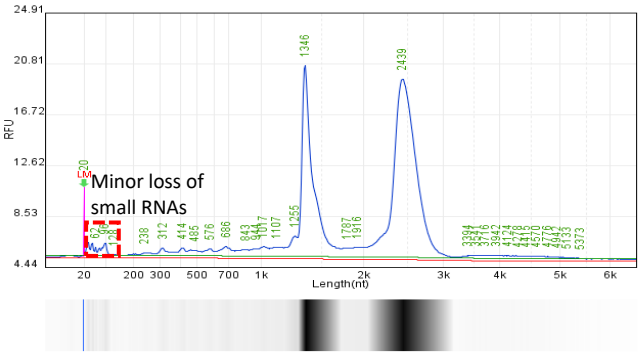
- Place the dried RB Column in a clean 1.5 ml microcentrifuge tube.
- Add **50 µl of RNase-free Water** into the CENTER of the RB Column.
- Incubate for 3 minutes at room temperature.
- Centrifuge at 14-16,000 x g for 2 minutes to recover the miRNA. Store the purified miRNA at –70°C for long term storage is recommended.

Appendix A: Optional Large RNA Purification Protocol

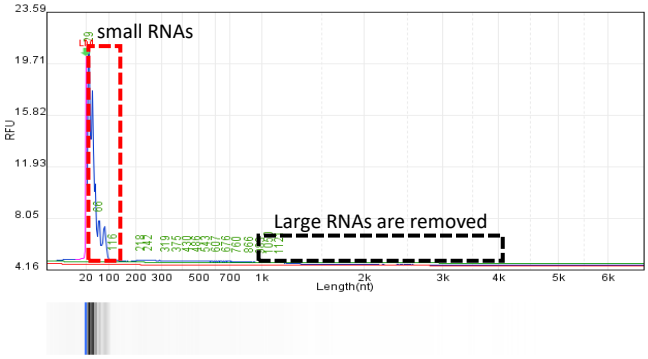
- Place the first RB column from Step 2 back in the 2 ml Collection Tube for large RNA fragments purification.
- Add **400 µl of W1 Buffer** into the RB Column then centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through then place the RB Column back in the 2 ml Collection Tube.
- Add **600 µl of Wash Buffer (make sure ethanol was added)** into the RB Column.
- Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.
- Place the RB Column back in the 2 ml Collection Tube.
- Add **600 µl of Wash Buffer (make sure ethanol was added)** into the RB Column.
- Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.
- Place the RB Column back in the 2 ml Collection Tube and centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.
- Place the dried RB Column in a clean 1.5 ml microcentrifuge tube.
- Add **50 µl of RNase-free Water** into the CENTER of the RB Column.
- Incubate for 3 minutes at room temperature.
- Centrifuge at 14-16,000 x g for 2 minutes to recover the large RNA fragments. Store the purified RNA at –70°C for long term storage is recommended.

Presto™ miRNA Purification Kit Test Data

(A) Large RNA removal



(B) Small RNA enrichment



Efficient Removal of Large RNAs and Enrichment of Small RNAs. Different RNA species were isolated from *E. coli* using Geneaid Presto™ miRNA Purification Kit, and the purified RNAs were analyzed by capillary electrophoresis (BIOptic Qsep100). Figure (A) shows the large RNA fragments were removed by the first RNA columns. Figure (B) shows the small RNA molecules that were isolated and enriched using the second RNA columns and shows that there is no contamination with any large RNA fragments. This demonstrates the effective separation of the small RNA from the large RNA species using Geneaid Presto™ miRNA Purification Kit.

Troubleshooting

Low RNA Yield

Insufficient disruption and homogenization.

- Ensure that the appropriate volume of PMI Buffer was used for the sample homogenization.
- Do not exceed the recommended amounts of starting materials.

RNA column has become clogged.

- Do not exceed the recommended amounts of starting materials.
- The sample lysate should be sheared by passing lysate through a 20-G needle syringe 10 times.
- Centrifugation temperature was too low (should be 20ºC to 25ºC).

Wrong operation with the flow-through from the first binding step.

- The flow-through from the binding step with the first RNA column contains the small RNA molecules. **DO NOT** discard the flow-through.
- Ensure that the appropriate volume of ethanol was added to the flow-through from the first binding step before it is applied to the second RNA column.

Inappropriate preparation with the Wash Buffer.

- Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Eluted DNA Does Not Perform Well In Downstream Applications

Trace of salts contamination.

- Wash the second RNA column with appropriate volume of Wash Buffer (make sure ethanol was added) twice.

Residual ethanol contamination.

- After wash steps, centrifuge the RNA column at 16,000 x g for additional 3 minutes to remove the residue ethanol.

Genomic DNA contamination.

- Too much amount of starting materials were used. Perform RNase-free DNase I digestion after elution to remove DNA contamination.

RNA is Degraded

Inappropriate handling of starting material.

- Tissue samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer. Do not allow frozen tissues to thaw prior sample homogenization.
- In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly.

Improper storage of the purified RNA.

- Store the purified RNA samples at -70°C for long term storage is recommended.