ISOSPIN Liquid Sample miRNA

Code No. 318-09191
Manual Ver. 1

Introductions:

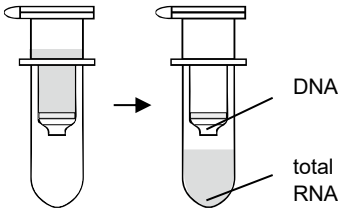
ISOSPIN Liquid Sample miRNA is a kit for purifying small RNAs, such as microRNAs (miRNAs), from liquid samples.

Features

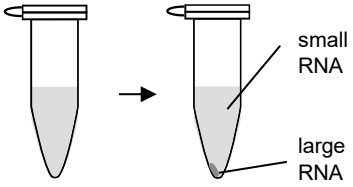
・ Small RNA can be purified from plasma, serum, whole blood, saliva, urine, and other liquid samples

Flow of small RNA purification

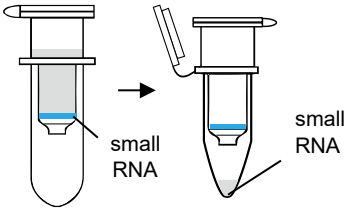
Step 1. Removal of DNA



Step 2. Removal of large RNA



Step 3. Purification of small RNA



Kit Contents:

The materials provided are sufficient for 50 preparations.

Component	Quantity	Storage
Proteinase K	1 ml x 1	-20˚C*1
LR Extraction Buffer	18 ml x 1	Room temp.
LR Wash1 Buffer	60 ml x 1	Room temp.*2
LR Wash2 Buffer	60 ml x 1	Room temp.*2
ddWater	1 ml x 5	Room temp.
Spin Column	50 pieces*3	Room temp.
Spin Column Blue	50 pieces*3	Room temp.

*1 This kit is shipped at room temperature. After arriving, Proteinase K should be stored at –20°C for long periods of time.

*2 LR Wash1 Buffer and LR Wash2 Buffer contain ethanol. Always keep buffer bottles tightly closed.

*3 A Spin Column consists of a column pre-inserted into a collection tube.

Required Materials Not Included:

Ethanol (96-100 %)
Water (RNase free)
Pipettors and pipette tips
Centrifuge for spin down
Microcentrifuge capable of centrifuging 13,000 x g at room temperature
Heat blocks or water baths set to 37°C
Vortex mixer
1.5 ml microcentrifuge tubes
2.0 ml microcentrifuge tubes, if necessary

* Use RNase free reagents and tubes.
* We recommend using nucleic acid low binding tubes.

Safety Data Sheet:

The Safety Data Sheets (SDSs) are available on our website at **www.nippongene.com**.

Method

Protocol A (Step 1, Step2, Step3)

Subjects: Liquid samples containing large RNA and small RNA

See P4.

Protocol B (Step 1, Step3)

Subjects: Liquid samples that do not contain large RNA, such as plasma or serum

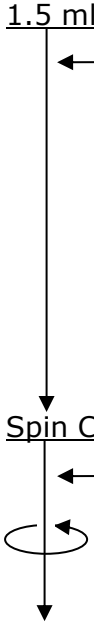
See P6.

NOTE: Please use fresh samples. Do not use samples that have been through multiple freeze-thaw cycles.

Protocol A (Step 1, Step2, Step3)

Subjects: Liquid samples containing large RNA and small RNA

Step 1: Removal of DNA



1.5 ml tube

1) Add 180 µl liquid sample, 20 µl Proteinase K, and 180 µl LR Extraction Buffer into the tube.

   Mix well by vortexing for 15 seconds.

2) Incubate the tube at 37°C for 15 minutes. During incubation, mix by vortexing every 5 minutes.

3) Spin down the tube.

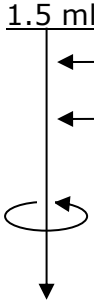
Spin Column

4) Apply all 380 µl of the mixture into a Spin Column.

5) Centrifuge at 13,000 x g for 15 seconds at room temperature to collect the flow-through.

Proceed to the next step.

Step 2: Removal of large RNA



1.5 ml tube

6) Transfer the flow-through to a clean 1.5 ml microcentrifuge tube.

7) Add 190 µl ethanol into the tube.
   Mix well by vortexing for 15 seconds.

8) Centrifuge at 13,000 x g for 15 minutes at room temperature to pellet large RNA.

Proceed to the next step.

Step 3-1: Purification of small RNA

1.5 ml tube

9) ← Transfer up to 570µl of the supernatant to a clean 1.5 ml microcentrifuge tube.

10) ← Add 380 µl ethanol into the tube.
Mix well by vortexing for 15 seconds.
Spin down the tube.

Spin Column Blue

11) ← Apply all (up to 950 µl) the mixture into a Spin Column Blue.

12) ↻ Centrifuge at 13,000 x g for 15 seconds at room temperature to bind small RNA to the column.

13) Remove the column from the collection tube and discard flow-through. Re-insert the column in the same collection tube.

14) ← Apply 500 µl LR Wash1 Buffer into the column.

15) ↻ Centrifuge at 13,000 x g for 15 seconds at room temperature.

16) Remove the column from the collection tube and discard flow-through. Re-insert the column in the same collection tube.

17) ← Apply 500 µl LR Wash2 Buffer into the column.

18) ↻ Centrifuge at 13,000 x g for 2 minutes at room temperature.

19) Discard flow-through and the collection tube. Avoid splashing any flow-through on the column.

Proceed to the next step.

Step 3-2: Elution of small RNA

20) Insert the column into a clean 1.5 ml microcentrifuge tube.

21) ← Apply 50 µl of ddWater to the center of column.
Incubate for 3 minutes at room temperature.

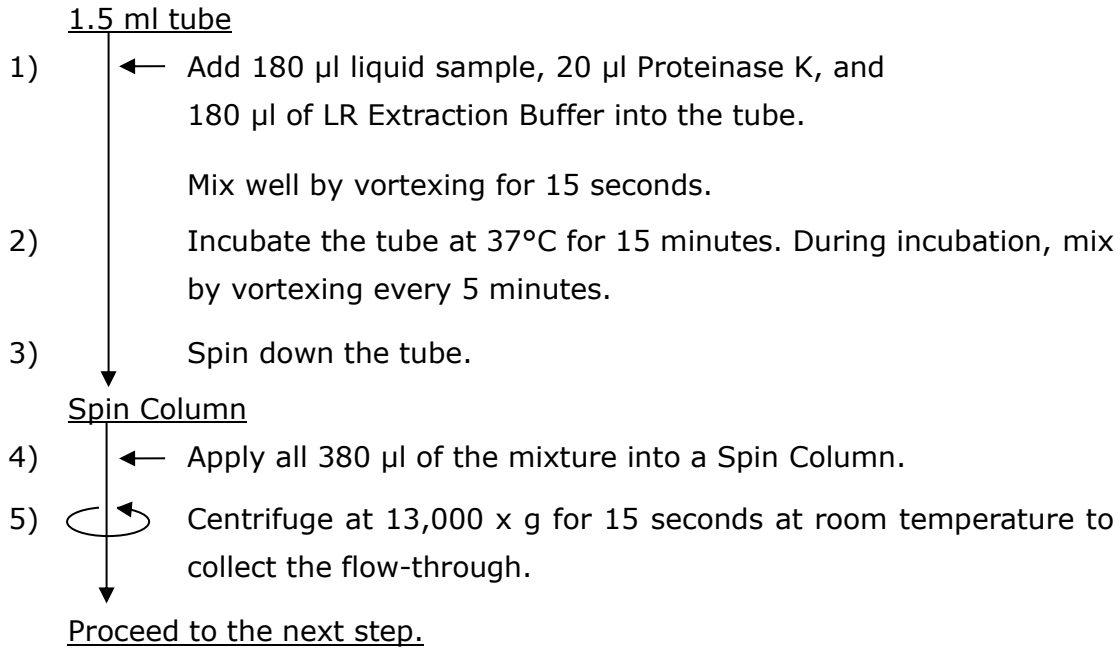
22) ↻ Centrifuge at 13,000 x g for 2 minutes at room temperature to elute small RNA.

23) Small RNA solution is collected in the tube.

Protocol B (Step 1, Step3)

Subjects: Liquid samples that do not contain large RNA, such as plasma or serum

Step 1: Removal of DNA



Step 2: Removal of large RNA *(SKIP)*

Step 3-1: Purification of small RNA

<u>1.5 ml tube</u>

6) Transfer the flow-through to a clean 1.5 ml microcentrifuge tube.

7) ← Add 570 µl ethanol into the tube.
Mix well by vortexing for 15 seconds.
Spin down the tube.

<u>Spin Column Blue</u>

8) ← Apply all 950 µl of the mixture into a Spin Column Blue.

9) Centrifuge at 13,000 x g for 15 seconds at room temperature to bind small RNA to the column.

10) Remove the column from the collection tube and discard flow-through. Re-insert the column in the same collection tube.

11) ← Apply 500 µl LR Wash1 Buffer into the column.

12) Centrifuge at 13,000 x g for 15 seconds at room temperature.

13) Remove the column from the collection tube and discard flow-through. Re-insert the column in the same collection tube.

14) ← Apply 500 µl LR Wash2 Buffer into the column.

15) Centrifuge at 13,000 x g for 2 minutes at room temperature.

16) Discard flow-through and the collection tube. Avoid splashing any flow-through on the column.

<u>Proceed to the next step.</u>

Step 3-2: Elution of small RNA

17) <u>Insert the column into a clean 1.5 ml microcentrifuge tube.</u>

18) ← Apply 50 µl of ddWater to the center of column.
Incubate for 3 minutes at room temperature.

19) Centrifuge at 13,000 x g for 2 minutes at room temperature to elute small RNA.

20) <u>Small RNA solution is collected in the tube.</u>

Troubleshooting:

Observation	Potential cause	Suggested action
DNA contamination	The amount of DNA in the sample is high.	The purified RNA solution is treated with DNase.
	DNA and RNA form a complex.	Following a 15 min incubation at 37˚C in Step 1-2) of the protocol, optionally, add an additional incubation at 80˚C for 5 minutes and on ice for 5 minutes.

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