ReliaPrep™ miRNA Cell and Tissue Miniprep System

Instructions for Use of Products Z6210, Z6211 and Z6212.

Promega

Quick Protocol

RNA Isolation and Purification Procedure from Cell Samples

Use the following protocol to lyse cultured cells from suspension. Use 1×10^2 to 1×10^6 cells per purification.

1. Collect cells by centrifugation at 300 × *g* for 5 minutes. Wash the cell pellet with ice-cold, sterile 1X PBS. Centrifuge at 300 × *g* for 5 minutes to collect the cells. Discard the supernatant.
2. Add 200µl of LBA + TG Buffer to the washed cell pellet. Mix well by vortexing and/or pipetting.

   Note: Following lysis, pipet 7–10 times to shear the DNA using a P200 or P1000 pipettor.
3. Add 130µl of RDB to each homogenate and vortex for 10 seconds. Centrifuge for 2 minutes at 12,000 × *g*. Carefully transfer the cleared homogenate to a clean 1.5ml tube.
4. Add 400µl of 100% isopropanol to each cleared homogenate. Mix by vortexing.
5. Transfer the homogenate to a ReliaPrep™ Minicolumn. Centrifuge at 12,000 × *g* for 30 seconds.
6. Remove the column and discard the liquid. Place the column back into the Collection Tube.
7. Transfer the remaining homogenate to the same column used in Step 5. Centrifuge at 12,000 × *g* for 30 seconds.
8. Remove the column and discard the liquid. Place the column back into the Collection Tube.
9. Add 500µl of RWA to each column. Centrifuge at 12,000 × *g* for 30 seconds.
10. Remove the column and discard the liquid. Place the column back into the Collection Tube.
11. Add 500µl of RWA to each column. Centrifuge at 12,000 × *g* for 2 minutes. Carefully transfer the column to a 1.5ml Elution Tube.
12. Add 40µl of Nuclease-Free Water to each column. Centrifuge at 12,000 × *g* for 1 minute.
13. Transfer 5µl of DNase 10X Buffer and 5µl of DNase I to eluate.
14. Incubate for 5 minutes at room temperature (20–25°C).
15. Add 150µl of LBA + TG Buffer to the DNase treatment tube.
16. Add 300µl of 95% ethanol to the mixture and vortex for 10 seconds. Transfer 500µl of this mixture to a new column. Centrifuge at 12,000 × *g* for 30 seconds.
17. Remove the column and discard the liquid. Place the column back into the Collection Tube and repeat Steps 9–11.
18. Add 15µl of Nuclease-Free Water to each column (see Table 1). Centrifuge at 12,000 × *g* for 1 minute.

Table 1. Recommended RNA Elution Volumes per Number of Cells.

Cell Input Range	Nuclease-Free Water
1×10^2 to 5×10^5	15µl
$>5 \times 10^5$ to 1×10^6	30µl

Note: RNA concentration may increase with lower elution volumes; however, the total yield of RNA may decrease when elution volumes are between 10–15µl. If maximum recovery of RNA is essential, we recommend a second elution into a second sterile tube with an additional 15µl of Nuclease-Free Water followed by centrifugation at 12,000 × *g* for 1 minute.

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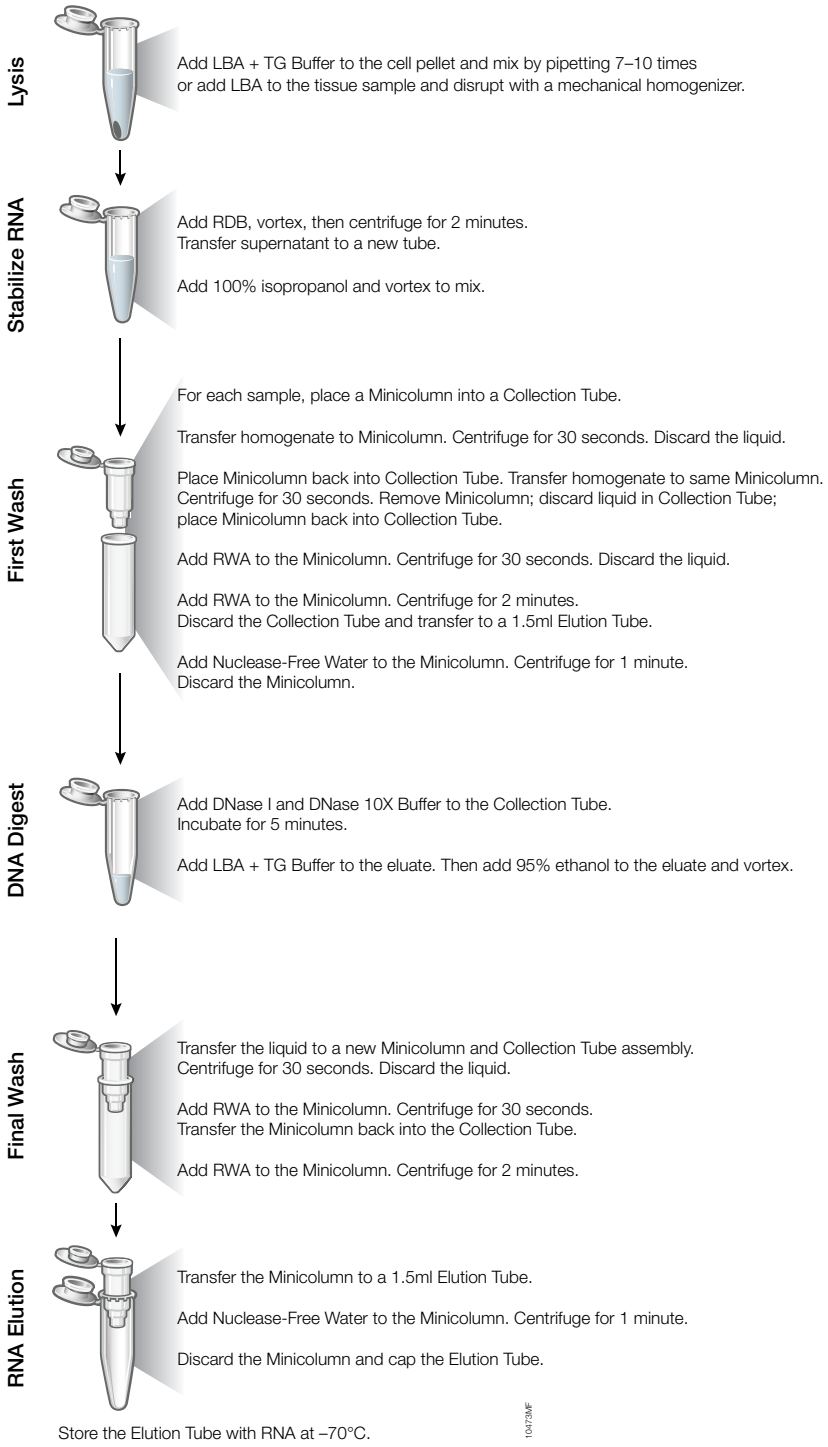

Figure 1. Schematic diagram of the ReliaPrep™ miRNA Cell and Tissue Miniprep System.

Additional protocol information is in Technical Manual #TM469, available online at: **www.promega.com**

