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 Tissue Samples

Use the following protocol to lyse tissue samples. Use 0.25–20mg of tissue per purification.

1. Place fresh or flash-frozen tissue samples in a sterile centrifuge tube containing 200µl LBA + TG. Use a mechanical homogenizer or mini pestle to disrupt the tissue until homogeneous.

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

Tissue Input Range	Nuclease-Free Water
0.25–5mg	15µl
>5–10mg	30µl
>10mg	50µl

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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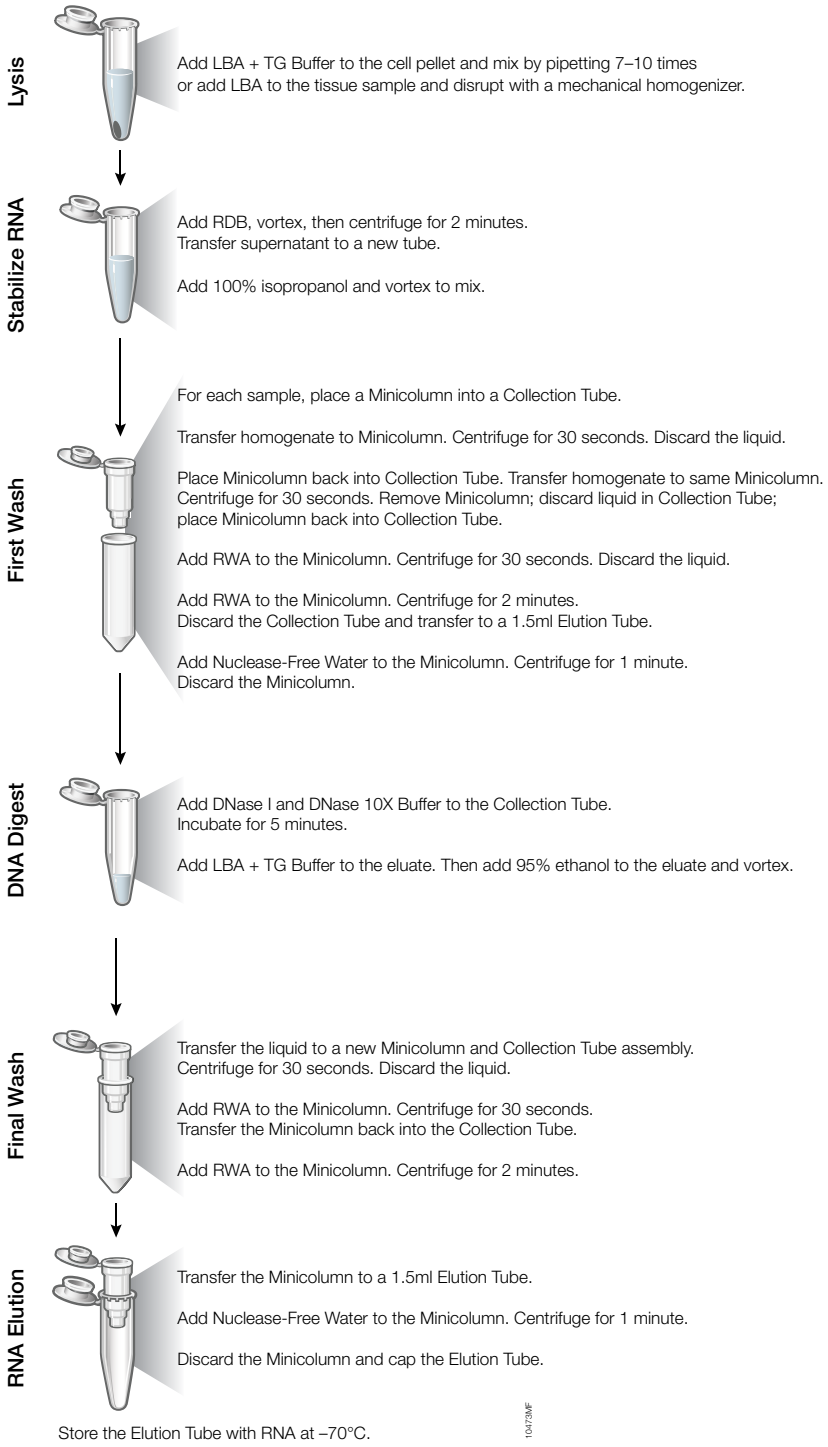

Figure 1. Schematic diagram of the ReliaPrep™ miRNA Cell and Tissue Miniprep System.

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

