Quick Protocol

# ReliaPrep™ RNA Miniprep System for Cells

Instructions for Use of Products **Z6014** and **Z6015**.

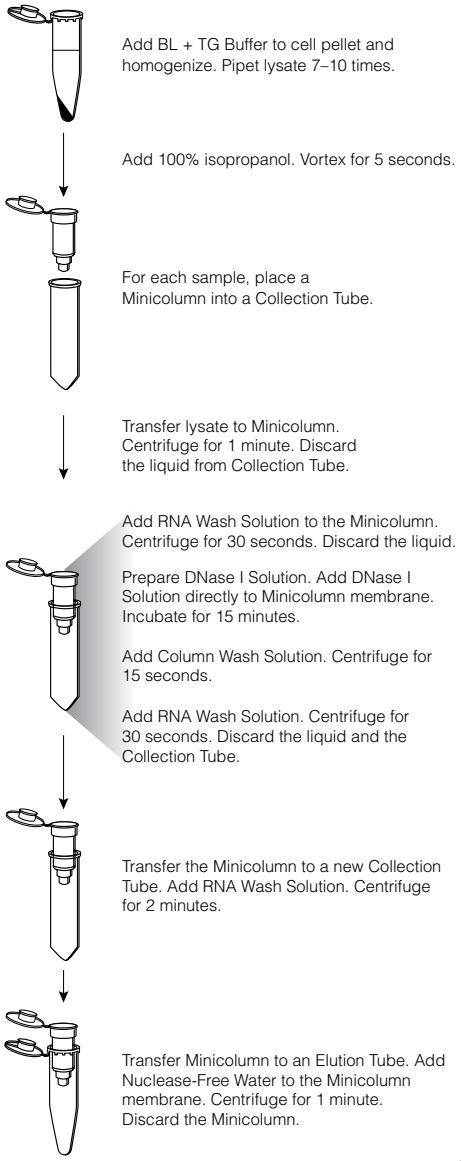
## Protocol

1. Before beginning the ReliaPrep™ RNA Miniprep for Cells protocol, see **Section 4.A., Preparing Solutions** in the *ReliaPrep™ RNA Miniprep System Technical Manual* #TM774. Prepare the four required solutions immediately prior to use, for best results.
2. To collect and lyse adherent cells, use the protocol in Section 4.B, TM774, and then proceed to Step 7. For suspension cells, proceed to Step 3.
3. Collect cells in a sterile centrifuge tube by centrifugation at 300 × *g* for 5 minutes.
4. Wash the cell pellet with ice-cold, sterile 1X PBS. Centrifuge at 300 × *g* for 5 minutes. Carefully discard the supernatant.
5. Add BL + TG Buffer to the washed cell pellet (see the table below). If frozen cell pellets are used as starting material, add BL + TG Buffer to the frozen pellets before thawing.

| Number of Cells | BL + TG Buffer | 100% Isopropanol |
|---|---|---|
| $1 \times 10^2$ to $5 \times 10^5$ | 100µl | 35µl |
| $>5 \times 10^5$ to $2 \times 10^6$ | 250µl | 85µl |
| $>2 \times 10^6$ to $5 \times 10^6$ | 500µl | 170µl |

6. Disperse the cell pellet and mix well by vortexing and/or pipetting.

   **Note:** After adding BL + TG Buffer, pipet 7–10 times to shear the DNA. For $>2 \times 10^6$ cells, pass the lysate through a 20-gauge needle four to five times to shear the genomic DNA.
7. Add isopropanol as recommended in the table above. Mix by vortexing 5 seconds.
8. Wearing gloves, unpack one Minicolumn, two Collection Tubes and one Elution Tube for each sample. Label each tube and Minicolumn. Place one Minicolumn into a Collection Tube for each sample.
9. Transfer lysate from Step 7 to a Minicolumn in a Collection Tube. Centrifuge at 12,000–14,000 × *g* for 30 seconds at 20°–25°C.
10. Remove the ReliaPrep™ Minicolumn and discard liquid in the Collection Tube. Replace the Minicolumn in the Collection Tube. Add **500µl of RNA Wash Solution** to the Minicolumn. Centrifuge at 12,000–14,000 × *g* for 30 seconds. Empty the Collection Tube.




Additional protocol information is in Technical Manual #TM774, available online at: **www.promega.com**

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11. Prepare **DNase I incubation mix** by combining the following amounts of reagent, per sample, **in the order listed**:

| Solution | Volume × Number of Preps = Total |
|---|---|
| Yellow Core Buffer | 24µl |
| $MnCl_2$, 0.09M | 3µl |
| DNase I | 3µl |

Mix by gently pipetting; **do not vortex**.The volumes listed above make enough DNase I mix for a single sample. Multiply this amount by the number of samples to calculate the amount of DNase I mix to prepare.

12. Apply **30µl of DNase I incubation mix** to the Minicolumn membrane. Incubate for 15 minutes at 20–25°C.
13. Add **200µl of Column Wash Solution** (with ethanol added) to the Minicolumn. Centrifuge at 12,000–14,000 × *g* for 15 seconds.
14. Add **500µl of RNA Wash Solution** (with ethanol added). Centrifuge at 12,000–14,000 × *g* for 30 seconds. Discard the wash solutions and the Collection Tube.
15. Place the ReliaPrep™ Minicolumn into a new Collection Tube. Add **300µl of RNA Wash Solution** and centrifuge at high speed for 2 minutes.
16. Transfer the ReliaPrep™ Minicolumn from the Collection Tube to an Elution Tube. Add **Nuclease-Free Water** to the Minicolumn membrane as recommended in the table below. Place the Minicolumn and Elution Tube into a centrifuge with the Elution Tube lid facing to the outside. Centrifuge at 12,000–14,000 × *g* for 1 minute.

| Number of Cells | Nuclease-Free Water |
|---|---|
| $1 \times 10^2$ to $5 \times 10^5$ | 15µl |
| $>5 \times 10^5$ to $2 \times 10^6$ | 30µl |
| $>2 \times 10^6$ to $5 \times 10^6$ | 50µl |

17. Discard the Minicolumn. Cap the Elution Tube containing the purified RNA and store at –70°C.

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