ReliaPrep™ RNA Cell Miniprep System

INSTRUCTIONS FOR USE OF PRODUCTS Z6010, Z6011 AND Z6012.

Quick PROTOCOL

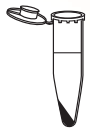
Protocol

1. Before beginning the ReliaPrep™ RNA Cell Miniprep protocol, see **Section 4.A., Preparation of Solutions** in the *ReliaPrep™ RNA Cell Miniprep System Technical Manual*, #TM370. Prepare the four required solutions immediately prior to use, for best results.
2. To harvest adherent cells, use the protocol in Section 8.A., TM370, prior to cell lysis. For suspension cells proceed to Step 3, below.
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
1×10^2 to 5×10^5	100µl	35µl
$>5 \times 10^5$ to 2×10^6	250µl	85µl
$>2 \times 10^6$ to 5×10^6	500µl	170µl

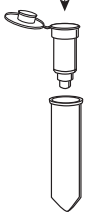
6. Disperse the cell pellet and mix well by vortexing and/or pipetting.

   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 4–5 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.



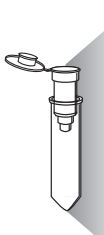
Add BL + TG Buffer to cell pellet and mix well by vortexing or pipetting. (To harvest adherent cells, see Section 8.A.)

Add 100% isopropanol. Vortex for 5 seconds.



For each sample, place a Minicolumn into a Collection Tube.

Transfer lysate to Minicolumn. Centrifuge for 30 seconds. Discard the liquid from Collection Tube.

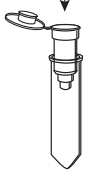


Add RNA Wash Solution to the Minicolumn. Centrifuge for 30 seconds. Discard the liquid.

Prepare DNase I Solution. Add DNase I Solution directly to Minicolumn membrane. Incubate for 15 minutes.

Add Column Wash Solution. Centrifuge for 15 seconds.

Add RNA Wash Solution. Centrifuge for 30 seconds. Discard the liquid and the Collection Tube.



Transfer the Minicolumn to a new Collection Tube. Add RNA Wash Solution. Centrifuge for 2 minutes.

Transfer Minicolumn to an Elution Tube. Add Nuclease-Free Water to the Minicolumn membrane. Centrifuge for 1 minute. Discard the Minicolumn.

Store the Elution Tube with RNA at –70°C.

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Protocol (continued)

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
1×10^2 to 5×10^5	15µl
$>5 \times 10^5$ to 2×10^6	30µl
$>2 \times 10^6$ to 5×10^6	50µl

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

Detailed protocol information is available in the *ReliaPrep™ RNA Cell Miniprep System Technical Manual* #TM370, available at:
www.promega.com/protocols/

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