PureYield™ RNA Midiprep System

INSTRUCTIONS FOR USE OF PRODUCTS Z3740, Z3741 AND Z3742.

Quick PROTOCOL

Lysate Preparation

These instructions describe lysate preparation from tissue samples and cultured cells. For information about lysate preparation from other samples, such as bacteria, yeast, plant tissues or blood, see the *PureYield™ RNA Midiprep System Technical Manual* #TM279. Technical literature is available online at: **www.promega.com/tbs/**

Preparing Lysates from Tissue Samples

1. Transfer 2ml of ice-cold Lysis Solution containing β-mercaptoethanol to a labeled tube.
2. Record the weight of the tube containing the Lysis Solution.
3. Excise the tissue of interest, and place it in the tube containing the Lysis Solution. Homogenize the tissue until no visible tissue fragments remain.
4. Weigh the tube containing the lysate. Calculate the approximate tissue mass, and adjust the lysate concentration with Lysis Solution containing β-mercaptoethanol if necessary (Table 1). Incubate the lysates on ice for 10 minutes to complete the lysis.

Table 1. Recommended Maximum Sample Amounts for Preparing Lysates.

Sample Type	Maximum Lysate Concentration per Milliliter of Lysis Solution
Liver	150mg/ml
Kidney	100mg/ml
Muscle	150mg/ml
Spleen	75mg/ml
Heart	150mg/ml
Brain	150mg/ml
Lung	150mg/ml

Preparing Lysates from Cultured Cells

1. Harvest the cultured cells by trypsinization or scraping. For suspension cells proceed to Step 2.
2. Centrifuge 1–5 × 10^7 cells in a sterile 50ml conical centrifuge tube at 300 × *g* for 5 minutes (4–23°C). Gently wash the cell pellet with 25ml of sterile, ice-cold 1X PBS. Centrifuge at 300 x *g* for 5 minutes. Immediately discard the supernatant, and place the sample on ice.
3. Add 2ml of ice-cold Lysis Solution containing β-mercaptoethanol. Vortex the sample; if clumps are visible, homogenize the lysate using a homogenizer at high speed. Incubate the lysates on ice for 10 minutes to complete the lysis.

Lysate Clearing

1. Transfer 2ml of the lysate prepared above to a 15ml centrifuge tube. If the volume of lysate is less than 2ml, add an appropriate volume of Lysis Solution containing β-mercaptoethanol to achieve 2ml.
2. Add 4ml of RNA Dilution Buffer. Seal the tube, and mix thoroughly by inverting the tube 3–4 times, then vortexing.
3. Add 1ml of thoroughly mixed Clearing Agent to the diluted lysate mixture. Mix by inverting 2–3 times, and vortex until homogeneous.
4. Incubate at 70°C for 5 minutes to denature the samples.
5. Remove the tubes, and cool at room temperature for at least 5 minutes.
6. Place one blue PureYield™ Clearing Column for each sample in a 50ml collection tube. Save the collection tube caps.
7. Mix each sample by vortexing or vigorously shaking until homogeneous. Immediately pour the mixture into the assembled PureYield™ Clearing Column/collection tube.
8. Centrifuge the PureYield™ Clearing Column assembly in a swinging bucket rotor at 2,000 × *g* at 22–25°C for 10 minutes to clear the lysate.
9. Discard the blue Clearing Column, and **save** the cleared lysate in the collection tube.

For additional information. see the PureYield™ RNA Midiprep System Technical Manual #TM279, available online at: **www.promega.com/tbs**

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Printed in USA, Revised 3/09
Part# 9FB084

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RNA Purification by Centrifugation (Spin)

1. Place a clear PureYield™ Binding Column into a 50ml collection tube for each sample.
2. Add 4ml of isopropanol to the cleared lysate, and mix thoroughly by swirling or shaking. Immediately pour the mixture into the PureYield™ Binding Column/collection tube assembly. Cap the tube if desired.
3. Centrifuge at 2,000 × *g* for 10 minutes in a swinging bucket rotor.
4. Carefully remove the PureYield™ Binding Column from the collection tube, and discard the flowthrough. Return the Binding Column to the tube.
5. Add 20ml of RNA Wash Solution (containing ethanol) to each PureYield™ Binding Column. Cap the tube if desired. Centrifuge at 2,000 x *g* for 5 minutes.
6. Empty the Collection Tube. Repeat the wash with 10ml of RNA Wash Solution, increasing the centrifugation time to 10 minutes to empty the column and dry the Binding Membrane.

   Optional: To further reduce ethanol carryover, empty the collection tube and centrifuge the PureYield™ Binding Column for an additional 5 minutes at 2,000 × *g*.
7. Carefully transfer the PureYield™ Binding Column to a fresh 50ml collection tube.
8. Add 1ml of Nuclease-Free Water to the PureYield™ Binding Columns. Be careful to completely cover the surface of the membrane. If desired, cap the tube. Incubate at room temperature for 2 minutes. Centrifuge at 2,000 x *g* for 3 minutes.
9. Remove the PureYield™ Binding Columns, and discard. Store the purified RNA at –70°C.

RNA Purification by Vacuum

1. Attach a clear PureYield™ Binding Column for each sample to an open port on the vacuum manifold.
2. Add 4ml isopropanol to the cleared lysate, and mix thoroughly by swirling or shaking. Immediately pour the mixture into the PureYield™ Binding Column.
3. Close any unused vacuum ports. Apply a vacuum of at least 15 inches of mercury, and allow the mixtures to pass through the columns. As the columns empty, close the vacuum ports.
4. Open an unused port to vent the manifold, and turn off the vacuum source. Once the pressure has been released, close the unused port, and open the ports with PureYield™ Binding Columns attached.
5. Add 20ml of RNA Wash Solution (containing ethanol) to each PureYield™ Binding Column. Apply the vacuum, and allow the wash to pass through the PureYield™ Binding Columns.
6. Turn off the vacuum source, and open an unused port to vent the manifold. Once the pressure has been released, close the unused port, and open ports with PureYield™ Binding Columns attached. Repeat the wash with 10ml of RNA Wash Solution.
7. Once all of the PureYield™ Binding Columns are empty, open all of the ports with columns attached, and maintain the vacuum for 3 minutes.
8. Carefully transfer the PureYield™ Binding Columns to fresh 50ml collection tubes.
9. Add 1ml of Nuclease-Free Water to the columns. Be careful to completely cover the surface of the membrane. If desired, cap the tube. Incubate at room temperature for 2 minutes. Centrifuge at 2,000 × *g* for 3 minutes.
10. Remove the PureYield™ Binding Columns, and discard. Store the purified RNA at –70°C.

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