ReliaPrep™ FFPE Total RNA Miniprep System

Instructions for Use of Products Z1001 and Z1002.

Quick Protocol

RNA Isolation with Deparaffinization Using Mineral Oil

Materials to Be Supplied By the User

- 95–100% ethanol
- 100% isopropanol
- 80°C heat block
- 56°C heat block
- equivalent of ≤100µm tissue sections (see Technical Manual #TM353)

Notes:

- Add Blue Dye to the Lysis Buffer before starting the procedure (see Technical Manual #TM353).
- Add 95–100% ethanol to the 1X Wash Solution before starting the procedure.
- Perform all centrifugations at room temperature.
- Prepare the DNase treatment mix immediately before use, making only the amount of DNase treatment mix required.

Deparaffinization Using Mineral Oil

1. Add mineral oil to the sample:
   - For sections ≤50 microns, add 300µl of mineral oil.
   - For sections >50 microns, add 500µl of mineral oil.
2. Incubate at 80°C for 1 minute.
3. Vortex to mix.

Sample Lysis

1. Add 100µl of Lysis Buffer (with Blue Dye added) to the sample.
2. Centrifuge at 10,000 × *g* for 15 seconds. Two phases will be formed, a lower blue (aqueous) phase and an upper (oil) phase.
3. Add 10µl of Proteinase K directly to the lower blue phase and mix by pipetting.
4. Incubate at 56°C for 15 minutes.
5. Incubate at 80°C for 1 hour.
6. Place the tubes on ice for 1 minute to cool. Then place the tubes at room temperature for 2 minutes.

   Optional storage: After incubating at 80°C, samples may be stored overnight at 2–10°C. After storage, allow them to warm to room temperature prior to adding DNase and proceeding with the protocol.

DNase Treatment

1. Add 30µl of DNase treatment mix directly to the lower blue phase of the sample. Mix by gentle pipetting.
2. Incubate at room temperature (20–25°C) for 15 minutes.

Additional protocol information is in Technical Manual #TM353, available online at: **www.promega.com**

RNA Isolation with Deparaffinization Using Mineral Oil (continued)

Nucleic Acid Binding

1. Add 325µl of BL Buffer to the lysed sample.
2. Add 200µl of isopropanol (100%). Vortex briefly to mix.
3. Centrifuge at 10,000 × *g* for 15 seconds. Two phases will be formed, a lower blue (aqueous) phase and an upper (oil) phase.
4. For each sample to be processed, place a Binding Column into one of the Collection Tubes provided.

   Note: Wear gloves when handling the columns and tubes.
5. Transfer the entire lower (aqueous) phase of the sample to the Binding Column/Collection Tube assembly, and cap the column. Discard the remaining mineral oil.

   Note: The mineral oil is inert and will not interfere with the extraction procedure if some of the oil phase is carried over to the Binding Column.
6. Centrifuge the assembly at 10,000 × *g* for 30 seconds.
7. Discard the flowthrough, and reinsert the column into the Collection Tube.
8. Proceed immediately to Column Washing and Elution.

Column Washing and Elution

1. Add 500µl of 1X Wash Solution (with ethanol added) to the Binding Column. Cap the column.
2. Centrifuge at 10,000 × *g* for 30 seconds.
3. Discard the flowthrough, and reinsert the column into the same Collection Tube.
4. Add 500µl of 1X Wash Solution to the Binding Column. Cap the column.
5. Centrifuge at 10,000 × *g* for 30 seconds.
6. Discard the flowthrough, and reinsert the Binding Column into the same Collection Tube.
7. Close the cap on the Binding Column, and centrifuge the Binding Column/Collection Tube assembly at 16,000 × *g* for 3 minutes to dry the column.

   Note: It is important to dry the column to prevent carryover of ethanol to the eluate.
8. Transfer the Binding Column to a clean Elution Tube (provided), and discard the Collection Tube.
9. Add 30–50µl of Nuclease-Free Water to the column, and cap the column.
10. Centrifuge at 16,000 × *g* for 1 minute. Remove and discard the Binding Column.
11. Cap the Elution Tube, and store the eluted RNA at –30 to –10°C or <–65°C.

Additional protocol information in Technical Manual #TM353, available online at: **www.promega.com**

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