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
- Tissue sections (as described in Technical Manual #TM353)

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 to the sample.
2. Spin at 10,000 × *g* for 15 seconds. Two phases will be formed, a lower (aqueous) phase and an upper (oil) phase.
3. Add 10µl Proteinase K directly to the lower phase; mix the lower phase by pipetting.
4. Incubate at 56°C for 15 minutes.
5. Incubate at 80°C for 15 minutes.
6. Remove the tubes from the 80°C heat block, and place them on ice for 1 minute to cool, then place tubes at room temperature for 2 minutes.

DNase Treatment

While the samples are equilibrating to room temperature, prepare the DNase treatment mix.

1. For each isolation to be performed, combine the following ingredients:

   13µl $MnCl_2$, 0.09M; 7µl DNase Buffer; 10µl DNase I Enzyme

   Notes:
   - Prepare only the amount of DNase treatment mix required.
   - Thaw and keep the DNase I enzyme on ice during use.
   - The DNase I enzyme is sensitive to physical inactivation. Mix by gentle pipetting. Do not vortex.
   - Prepare the DNase treatment mix immediately before use. The components of the DNase treatment mix should be stored separately and mixed fresh for each set of RNA extractions.
2. Add 30µl of freshly prepared DNase treatment mix directly to the lower phase of the sample; mix by gently pipetting.
3. Incubate for 15 minutes at room temperature (20–25°C).

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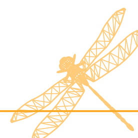

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RNA Isolation with Deparaffinization Using Mineral Oil (continued)

Nucleic Acid Binding

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

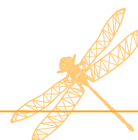
   Note: Wear gloves when handling the columns and tubes.
6. Transfer the entire lower (aqueous) phase of the sample to the Binding Column/Collection Tube assembly. Cap the column. Discard the mineral oil (upper phase).
7. Spin the assembly at 10,000 × *g* for 30 seconds.
8. Discard the flowthrough, and reinsert the Binding Column into the Collection Tube.
9. 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. Spin at 10,000 × *g* for 30 seconds.
3. Discard the flowthrough, and reinsert the Binding Column into the same Collection Tube used in Nucleic Acid Binding.
4. Add 500µl of 1X Wash Solution (with ethanol added) to the Binding Column. Cap the column.
5. Spin at 10,000 × *g* for 30 seconds.
6. Discard the flowthrough, and reinsert the Binding Column into the Collection Tube used for the Nucleic Acid Binding.
7. Close the cap on the Binding Column, and spin 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).
9. Add 30–50µl of Nuclease-Free Water to the column and cap the column.
10. Spin at 16,000 × *g* for 1 minute. Remove and discard the Binding Column.
11. Cap the Elution Tube, and store the eluted RNA at –20°C or –70°C.

For a detailed protocol and additional information please see Technical Manual #TM353, available at: www.promega.com



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