# ReliaPrep™ FFPE gDNA Miniprep System

INSTRUCTIONS FOR USE OF PRODUCTS A2351 AND A2352.

Quick PROTOCOL

## DNA Isolation with Deparaffinization Using Mineral Oil

### Materials to Be Supplied By the User

- 95–100% ethanol
- 80°C heat block
- 56°C heat block
- equivalent of ≤100μm tissue sections (see Technical Manual #TM352)

Note: All centrifugations are performed at room temperature.

### 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 200μl 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 20μl of Proteinase K directly to the lower phase; mix the lower phase by pipetting.
4. Incubate at 56°C for 1 hour.
5. Incubate at 80°C for 1 hour.
6. Allow the sample to cool to room temperature. Centrifuge briefly to collect any drops from the inside of the lid.

### RNase Treatment

1. Add 10μl of RNase A directly to the lysed sample in the lower phase. Mix the lower phase by pipetting.
2. Incubate at room temperature (20–25°C) for 5 minutes.

**ORDERING/TECHNICAL INFORMATION:**
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

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## DNA Isolation with Deparaffinization Using Mineral Oil (continued)

### Nucleic Acid Binding

1. Add 220µl of BL Buffer to the lysed sample.
2. Add 240µl of ethanol (95–100%).
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, including any precipitate that may have formed, 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.
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.
4. Add 500µl of 1X Wash Solution (with ethanol added, see Section 3) 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. Open 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: Centrifuging with the cap open ensures thorough drying of the column. It is important to dry the column to prevent carryover of ethanol to the eluate.
8. Transfer the Binding Column to a clean 1.5ml microcentrifuge tube (not provided), and discard the Collection Tube.
9. Add 30–50µl of Elution Buffer 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 microcentrifuge tube, and store the eluted DNA at –20°C.

*For a detailed protocol and additional information please see Technical Manual #TM352, available at: www.promega.com/tbs*

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