ReliaPrep™ FFPE gDNA Miniprep System

Instructions for Use of Products A2351 and A2352.

Promega

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
- 1.5 or 2ml microcentrifuge tubes
- equivalent of ≤100µm tissue sections (see Technical Manual #TM352)

Notes:

- Add Blue Dye to the Lysis Buffer before starting the procedure (see Technical Manual #TM352).
- Add 95–100% ethanol to the 1X Wash Solution before starting the procedure.
- Perform all centrifugations 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 200ul 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 20µl of Proteinase K directly to the lower phase and mix by pipetting.
4. Incubate at 56°C for 1 hour.
5. Incubate at 80°C for 4 hours.

   Note: Optimal recovery of amplifiable DNA is obtained with a 4-hour incubation at 80°C. Incubation may be reduced to 1 hour, but it will result in lower DNA yields.
6. Allow the sample to cool to room temperature. Centrifuge briefly to collect any condensation.

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

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.

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%). 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 blue (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.
6. Centrifuge the assembly at 10,000 × *g* for 30 seconds.
7. Discard the flowthrough, and reinsert the Binding 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 Binding 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. Open 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: 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. Centrifuge at 16,000 × *g* for 1 minute. Remove and discard the Binding Column.
11. Cap the microcentrifuge tube, and store the eluted DNA at –30 to –10°C.

Additional protocol information in Technical Manual #TM352, available online at: **www.promega.com**

