



Promega

Technical Manual

ReliaPrep™ FFPE gDNA Miniprep System

INSTRUCTIONS FOR USE OF PRODUCTS A2351 AND A2352.



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ReliaPrep™ FFPE gDNA Miniprep System

All technical literature is available at: www.promega.com/protocols/
 Please visit the web site to verify that you are using the most current version of this Technical Manual. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com

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1. Description

Formalin fixation and paraffin embedding (FFPE) is a commonly used method for archiving tissue specimens. The ability to extract DNA from these samples provides the potential for correlating disease state and tissue morphology with genotype. Extraction of DNA from FFPE tissues historically has been a challenge because the formalin fixation process results in cross-linking between proteins and DNA, as well as between different strands of DNA (1). The nonoptimal preservation of genomic DNA in FFPE complicates its use in many standard downstream analysis applications.

The ReliaPrep™ FFPE gDNA Miniprep System uses incubation conditions optimized to partially reverse this cross-linking without the need for an overnight digestion. This system also incorporates a deparaffinization method that does not rely on xylene or other hazardous or volatile solvents.

Note: The cross-linking introduced by the formalin fixation and paraffin embedding process results in nucleic acids that are partially degraded. **The degree of DNA fragmentation will vary depending on sample type, the age and storage conditions of the sample as well as the conditions used during formalin fixation.** When designing downstream amplification assays, best results will be achieved when targeting regions of 200 nucleotides or less.

2. Product Components and Storage Conditions

Product	Size	Cat.#
ReliaPrep™ FFPE gDNA Miniprep System	10 reactions	A2351

For Research Use Only. Not for Use in Diagnostic Procedures.

Contains sufficient reagents to perform genomic DNA isolations from 10 FFPE samples. Includes:

- 5ml Mineral Oil
- 2 × 1ml Lysis Buffer (LBA)
- 250µl Proteinase K (PK)
- 3.25ml BL Buffer
- 3ml Wash Solution
- 200µl RNase A
- 15ml Elution Buffer
- 1 package Collection Tubes (10 tubes/package)
- 1 package ReliaPrep™ FFPE Binding Columns (10 columns/package)

Product	Size	Cat.#
ReliaPrep™ FFPE gDNA Miniprep System	100 reactions	A2352

For Research Use Only. Not for Use in Diagnostic Procedures.

Contains sufficient reagents to perform genomic DNA isolations from 100 FFPE samples. Includes:

- 50ml Mineral Oil
- 20ml Lysis Buffer (LBA)
- 2 × 1.1ml Proteinase K (PK)
- 32.5ml BL Buffer
- 30ml Wash Solution
- 1ml RNase A
- 15ml Elution Buffer
- 2 packages Collection Tubes (50 tubes/package)
- 2 packages ReliaPrep™ FFPE Binding Columns (50 columns/package)

Storage Conditions: Store at room temperature.

3. Preparation of 1X Wash Solution

Materials to Be Supplied by the User

- 95–100% ethanol

10-reaction size: Add 12ml of 95–100% ethanol to the bottle containing 3ml of concentrated Wash Solution.

100-reaction size: Add 120ml of 95–100% ethanol to the bottle containing 30ml of concentrated Wash Solution.

Note: After adding ethanol, mark on the bottle that you have performed this step. This reagent is stable at 22–25°C when capped tightly.

4. Preparation of FFPE Sections

Materials to Be Supplied by the User

- 1.5 or 2ml microcentrifuge tube

1. Using a sterile blade, trim excess paraffin off the tissue block.

2. Cut 5–50µm sections from FFPE blocks using a microtome.

Note: If you are extracting nucleic acid from tissue sections that have been applied to microscope slides, use a sterile blade to scrape the sections from the slide.

3. Place the sections in a 1.5 or 2ml microcentrifuge tube (not provided). The equivalent of ≤100µm of tissue slices may be processed per reaction.

4. Proceed immediately to Section 5, DNA Isolation.

5. DNA Isolation

Materials to Be Supplied by the User

- 95–100% ethanol
- 80°C heat block
- 56°C heat block
- 1.5 or 2ml microcentrifuge tubes

Note: We recommend deparaffinization of FFPE samples using mineral oil (provided). For other methods of deparaffinization, refer to Section 8, Alternative Methods of Deparaffinization.

5.A. 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.

5.B. Sample Lysis

1. Add 200 μ l of Lysis Buffer to the sample.
2. Spin at 10,000 \times g for 15 seconds at room temperature. Two phases will be formed, a lower (aqueous) phase and an upper (oil) phase.
3. Add 20 μ l 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 at room temperature to collect any drops from the inside of the lid.

5.C. RNase Treatment

1. Add 10 μ l 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.

5.D. Nucleic Acid Binding

1. Add 220 μ l of BL Buffer to the lysed sample.
2. Add 240 μ l of ethanol (95–100%).
3. Vortex briefly to mix.
4. Spin at 10,000 \times g for 15 seconds at room temperature. 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 \times g for 30 seconds at room temperature.
8. Discard the flowthrough, and reinsert the Binding Column into the Collection Tube.
9. Proceed immediately to Section 6, Column Washing and Elution.

6. Column Washing and Elution

1. Add 500 μ l of 1X Wash Solution (with ethanol added) to the Binding Column. Cap the column.
2. Spin at 10,000 $\times g$ for 30 seconds at room temperature.
3. Discard the flowthrough, and reinsert the Binding Column into the same Collection Tube used for the Nucleic Acid Binding (Section 5.D).
4. Add 500 μ l of 1X Wash Solution (with ethanol added, see Section 3) to the Binding Column. Cap the column.
5. Spin at 10,000 $\times g$ for 30 seconds at room temperature.
6. Discard the flowthrough, and reinsert the Binding Column into the Collection Tube used for the Nucleic Acid Binding (Section 5.D).
7. Open the cap on the Binding Column, and spin the Binding Column/ Collection Tube assembly at 16,000 $\times g$ for 3 minutes at room temperature 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 30–50 μ l of Elution Buffer to the column, and cap the column.
10. Spin at 16,000 $\times g$ for 1 minute at room temperature. Remove and discard the Binding Column.
11. Cap the microcentrifuge tube, and store the eluted DNA at -20°C .

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. E-mail: techserv@promega.com

<u>Symptoms</u>	<u>Causes and Comments</u>
Partially dissolved paraffin	Large amounts of paraffin may require addition of extra mineral oil to dissolve completely. Prior to deparaffinization, use a sterile blade to trim away excess paraffin that does not contain tissue.
Low DNA yield	<p>DNA yield may vary depending on tissue type; consider increasing the amount of starting material that is processed.</p> <p>DNA yield may vary depending on sample type, the age and storage conditions of the sample, and the conditions used during formalin fixation. This method of nucleic acid extraction has not been tested with tissues fixed by methods other than formalin.</p> <p>Ethanol was not added to the Wash Solution prior to use. Confirm that ethanol was added to the Wash Solution; record addition on the bottle label.</p>
DNA appears degraded	The cross-linking introduced by the formalin fixation and paraffin embedding process results in nucleic acids that are characteristically partially degraded. When designing downstream amplification assays, best results will be achieved when targeting regions of 200 nucleotides or less.
Downstream amplification reactions appear inhibited	Ethanol carryover in the eluted DNA sample can inhibit downstream enzymatic reactions. Prior to the final DNA elution step, confirm that the Binding Column is dried by spinning at maximum speed for 3 minutes. Dry the column with the cap open.
The lysate has not passed completely through the Binding Column after centrifugation	Large amounts of tissue can result in concentrated lysates. If the lysate has not passed completely through the Binding Column after centrifugation, repeat the centrifugation step, increasing the speed to 16,000 × g. Overloading the column can result in reduced yield; consider reducing the amount of starting material.

8. Alternative Methods of Deparaffinization

8.A. DNA Isolation with Deparaffinization Using Xylene

Materials to Be Supplied By the User

- 95–100% ethanol
- 100% xylene
- 80°C heat block
- 56°C heat block
- 37°C heat block

Note: Xylene is a hazardous chemical.

Deparaffinization

1. Add 1ml of 100% xylene to the sample.
2. Vortex to mix.
3. Spin for 2 minutes at maximum speed at room temperature.
4. Remove the xylene without disturbing the pellet.
5. Add 1ml of 95–100% ethanol.
6. Vortex to mix.
7. Spin for 2 minutes at maximum speed at room temperature.
8. Remove the supernatant without disturbing the pellet.
9. Centrifuge for 30 seconds at maximum speed to collect remaining drops of ethanol; remove as much residual ethanol as possible without disturbing the pellet using a fine pipette tip.
10. Dry pellet for 5–15 minutes at 37°C to evaporate residual ethanol.

Lysis

1. Resuspend the pellet in 200µl of Lysis Buffer.
2. Add 20µl of Proteinase K to the sample; vortex briefly to mix.
3. Incubate at 56°C for 1 hour.
4. Incubate at 80°C for 1 hour.
5. 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. Pipet to mix.
2. Incubate at room temperature (20–25°C) for 5 minutes.

8.A. DNA Isolation Using Xylene Deparaffinization (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. For each sample to be processed place a Binding Column into one of the Collection Tubes provided. Wear gloves when handling the columns and tubes.
5. Transfer the entire sample, including any precipitate that may have formed, to the Binding Column/Collection Tube assembly, and cap the column.
6. Spin the assembly at 10,000 × *g* for 30 seconds at room temperature.
7. Discard the flowthrough, and reinsert the Binding Column into the same Collection Tube.
8. Proceed immediately to Section 6, Column Washing and Elution.

8.B. DNA Isolation without Deparaffinization

Materials to Be Supplied By the User

- 80°C heat block
- 56°C heat block

Sample Lysis

1. Add 200µl of Lysis Buffer to the sample.
2. Incubate at 80°C for 1 minute.
3. Vortex to mix, then spin at maximum speed for 1 minute.
4. Add 20µl of Proteinase K directly to the aqueous portion of the sample; mix by pipetting.
5. Incubate at 56°C for 1 hour.
6. Incubate at 80°C for 1 hour.
7. 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. Mix by pipetting.
2. Incubate at room temperature (20–25°C) for 5 minutes.

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. For each sample to be processed place a Binding Column into one of the Collection Tubes provided. Wear gloves when handling the columns and tubes.
5. Transfer the entire aqueous portion of the sample to the Binding Column/Collection Tube assembly. Cap the column.
6. Spin the assembly at 10,000 × *g* for 30 seconds at room temperature.
7. Discard the flowthrough, and reinsert the Binding Column into the same Collection Tube.
8. Proceed immediately to Section 6, Column Washing and Elution.

9. References

1. Lin, J. *et al.* (2009) High-quality genomic DNA extraction from formalin-fixed and paraffin-embedded samples deparaffinized using mineral oil. *Anal Biochem.* **365**, 265–7.

10. Related Products

Nucleic Acid Purification Systems

Product	Size	Cat.#
ReliaPrep™ FFPE Total RNA Miniprep System*	10 reactions	Z1001
ReliaPrep™ gDNA Tissue Miniprep System*	100 preps	A2051
ReliaPrep™ Large Volume HT gDNA Isolation System	96 × 10ml preps	A1751
ReliaPrep™ Blood gDNA Miniprep System	100 preps	A5081
Maxwell® 16 FFPE Plus LEV DNA Purification Kit	48 preps	AS1135

*Additional Sizes Available.

Biochemical Reagents

Product	Size	Cat.#
Mineral Oil	12ml	DY1151
Nuclease-Free Water*	50ml (2 × 25ml)	P1193
RNase ONE™ Ribonuclease*	1,000u	M4261
Ribonuclease H*	50u	M4281

*Additional Sizes Available.

PCR Reagents

Product	Size	Cat.#
GoTaq® qPCR Master Mix	200 reactions	A6001
GoTaq® Hot Start Polymerase	100u	M5001
GoTaq® Hot Start Green Master Mix	100 reactions	M5122

Additional Sizes Available.

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