TECHNICAL MANUAL

ReliaPrep™ gDNA Tissue Miniprep System

Instructions for Use of Products
A2050, A2051 and A2052

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ReliaPrep™ gDNA Tissue Miniprep System

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

1. Description

The ReliaPrep™ gDNA Tissue Miniprep System[a] provides a fast, simple technique for purifying intact DNA from mouse tails, tissues and buccal swabs in as little as 30 minutes, depending on the number of samples processed. Samples are processed using a minicolumn in a benchtop microcentrifuge. Up to 25mg of tissue (mouse tail or animal tissue) or a single buccal swab can be processed per purification. The genomic DNA isolated with this system is of high quality and can be used in common applications such as agarose gel analysis, restriction enzyme digestion and PCR analysis.

The ReliaPrep™ gDNA Tissue Miniprep System uses a simple four-step method:

1. Disrupting or homogenizing the starting material to release DNA.
2. Binding DNA to the ReliaPrep™ Binding Column.
3. Removing impurities with wash solution.
4. Eluting purified DNA.

No ethanol is used in the purification protocol, eliminating downstream problems caused by ethanol carryover.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
ReliaPrep™ gDNA Tissue Miniprep System—Sample Size	**10 preps**	**A2050**

Each system contains sufficient reagents for 10 purifications. Includes:

- 1 pack ReliaPrep™ Binding Columns (10/pack)
- 1 pack Collection Tubes (40/pack)
- 3ml Cell Lysis Buffer (CLD)
- 1ml Tail Lysis Buffer (TLA)
- 250µl Proteinase K (PK) Solution
- 3ml Binding Buffer (BBA)
- 17ml Column Wash Solution (CWD)
- 13ml Nuclease-Free Water
- 250µl RNase A Solution (4mg/ml)

PRODUCT	SIZE	CAT.#
ReliaPrep™ gDNA Tissue Miniprep System	**100 preps**	**A2051**

Each system contains sufficient reagents for 100 purifications. Includes:

- 2 packs ReliaPrep™ Binding Columns (50/pack)
- 10 packs Collection Tubes (40/pack)
- 40ml Cell Lysis Buffer (CLD)
- 10ml Tail Lysis Buffer (TLA)
- 2 × 1.1ml Proteinase K (PK) Solution
- 50ml Binding Buffer (BBA)
- 165ml Column Wash Solution (CWD)
- 25ml Nuclease-Free Water
- 2.25ml RNase A Solution (4mg/ml)

PRODUCT	SIZE	CAT.#
ReliaPrep™ gDNA Tissue Miniprep System	**250 preps**	**A2052**

Each system contains sufficient reagents for 250 purifications. Includes:

- 5 packs ReliaPrep™ Binding Columns (50/pack)
- 5 packs Collection Tubes (200/pack)
- 100ml Cell Lysis Buffer (CLD)
- 25ml Tail Lysis Buffer (TLA)
- 5.5ml Proteinase K (PK) Solution
- 2 × 68.75ml Binding Buffer (BBA)
- 412.5ml Column Wash Solution (CWD)
- 2 × 25ml Nuclease-Free Water
- 6 × 1ml RNase A Solution (4mg/ml)

Storage Conditions: Store all components at 15–30°C.

3. Protocols

Materials to Be Supplied by the User

- homogenizer (optional)
- vortex mixer
- 1.5ml microcentrifuge tubes
- heating block set to 56°C
- microcentrifuge capable of 14,000 × *g*
- phosphate-buffered saline (PBS)

Notes:

1. We recommend homogenizing or disrupting the tissue as much as possible (e.g., slicing the tissue with a razor blade or scalpel, grinding it in liquid nitrogen or using a homogenizer). Alternatively, if there is a standard method that you have used previously or a known method that works best for your sample type, that method can be used with the ReliaPrep™ System. We recommend disrupting samples in no more than 160µl of phosphate-buffered saline. Note that intact tissue can be used in this protocol, but we recommend incubating tissue in lysis buffer for two hours. If samples are thoroughly homogenized, an incubation of 30 minutes should be sufficient.
2. Buccal swabs do not need to be homogenized. An incubation of 10–30 minutes at 56°C in Cell Lysis Buffer (CLD)/Proteinase K (PK) Solution will release the DNA.
3. For mouse tail clippings, use 0.5–1.2cm of tail taken from the tip. Samples further from the tip of the tail contain more cartilaginous material that will clog the minicolumn. Cut the tail into two equal parts, and place in a 1.5ml tube. Incubate samples in Proteinase K (PK) Solution and Tail Lysis Buffer (TLA) at 56°C for 1 hour to overnight. Use of a shaker/incubator will greatly help in releasing the DNA.

3.A. Protocol for Mouse Tail

1. Place a 0.5–1.2cm sample of mouse tail in a 1.5ml microcentrifuge tube.

   Note: The smaller the length of mouse tail, the more efficient the lysis will be. If processing more than 1cm of tail, cut it into smaller pieces with a razor blade or scalpel. (See Note 3 above.)
2. Add 100µl of Tail Lysis Buffer (TLA) and 20µl of Proteinase K (PK) Solution to the sample. Vortex for 10 seconds.
3. Cap the tube, and incubate at 56°C for 1 hour to overnight.

   Note: A shaker/incubator set at 56°C will make the lysis more effective. If you do not have one, vortex the sample for 10 seconds every 30 minutes.
4. Add 300µl of Cell Lysis Buffer (CLD) and 20µl of RNase A Solution to each sample, mix by vortexing for 10 seconds and place at 56°C for 10 minutes.

   Note: If the presence of RNA is not a concern, skip this step.

! Incubating longer than 10 minutes is unnecessary.

3.A. Protocol for Mouse Tail (continued)

5. Remove the tube from the heating block. Add 250µl of Binding Buffer (BBA), cap the tube and mix by vortexing for 10 seconds with a vortex mixer.

   Note: If large amounts of intact tissue are still visible in the tube, centrifuge the sample in a microcentrifuge for 1 minute to pellet the debris.

6. Place a ReliaPrep™ Binding Column inside a collection tube for each sample. Transfer the liquid portion of the sample onto the binding column, cap the column and place it in a microcentrifuge.

7. Centrifuge for 1 minute at maximum speed. Check the binding column to make sure that the lysate has completely passed through the membrane. If lysate is still visible on top of the membrane, centrifuge the column for another minute.

8. Remove the collection tube containing flowthrough, and discard the liquid as hazardous waste.

9. Place the binding column into a fresh collection tube. Add 500µl of Column Wash Solution (CWD) to the column, and centrifuge for 2 minutes at maximum speed. Discard the flowthrough.

   Note: If any wash solution remains on the membrane, centrifuge the column for another minute.

10. Repeat Step 9 twice for a total of three washes.

11. Place the column in a clean 1.5ml microcentrifuge tube.

12. Add 50–200µl of Nuclease-Free Water to the column. Centrifuge for 1 minute at maximum speed.

    Note: The lower the volume of eluant added, the higher the final DNA concentration will be. However, some DNA (15–20%) will be lost at the lower volumes.

13. Discard the ReliaPrep™ Binding Column, and save the eluate. Do not reuse binding columns or collection tubes. The genomic DNA can be placed at 4°C for short-term storage or –20°C for long-term storage.

3.B. Standard Protocol for Animal Tissue

1. Weigh out 25mg of sample.

   Note: Cut the sample into smaller pieces with a razor blade or scalpel before proceeding. If you wish to grind the tissue under liquid nitrogen, place sample into a chilled mortar and pestle, and grind under nitrogen until the tissue has a powder-like consistency.

2. Add 160µl of PBS to each sample to be processed, and mix by vortexing. If samples were ground under liquid nitrogen, add the PBS to the powdered sample.

3. Homogenize the sample with a Dounce or rotary homogenizer, or use a previously established homogenization method for your particular sample.

   Note: This step is not necessary for tissues that were ground under liquid nitrogen.

4. Add 20µl of Proteinase K (PK) Solution to the homogenized sample.

5. Add 200µl of Cell Lysis Buffer (CLD) to the tube. Cap and mix by vortexing for at least 10 seconds.

6. Incubate at 56°C for 30 minutes to 2 hours.

   Notes:

   1. Using a shaker/incubator set at 56°C will make lysis more effective. If one is not available, vortex the sample for 10 seconds every 30 minutes.
   2. If your samples are difficult to lyse or not completely homogenized, a longer incubation time may be required.

7. Add 20µl of RNase A Solution to each sample, mix by vortexing for 10 seconds and place microcentrifuge tube at 56°C for 10 minutes.

   Note: If the presence of RNA is not a concern, skip this step.

   Incubating longer than 10 minutes is unnecessary.

8. Remove the tube from the heating block. Add 250µl of Binding Buffer (BBA), cap the tube and mix by vortexing for 10 seconds with a vortex mixer.

   Note: If large amounts of intact tissue are still visible in the tube, centrifuge the sample in a microcentrifuge for 1 minute to pellet the debris.

9. Place a ReliaPrep™ Binding Column inside a collection tube for each sample. Transfer the liquid portion of the sample onto the binding column, cap the column and place it in a microcentrifuge.

10. Centrifuge for 1 minute at maximum speed. Check the binding column to make sure that the lysate has completely passed through the membrane. If lysate is still visible on top of the membrane, centrifuge the column for another minute.

11. Remove the collection tube containing flowthrough, and discard the liquid as hazardous waste.

12. Place the binding column into a fresh collection tube. Add 500µl of Column Wash Solution (CWD) to the column, and centrifuge for 2 minutes at maximum speed. Discard the flowthrough.

    Note: If any wash solution remains on the membrane, centrifuge the column for another minute.

13. Repeat Step 12 twice for a total of three washes.

14. Place the column in a clean 1.5ml microcentrifuge tube.

15. Add 50–200µl of Nuclease-Free Water to the column. Centrifuge for 1 minute at maximum speed.

    Note: The lower the volume of eluant added, the higher the final DNA concentration will be. However, some DNA (15–20%) will be lost at the lower volumes.

16. Discard the ReliaPrep™ Binding Column, and save the eluate. Do not reuse binding columns or collection tubes. The genomic DNA can be placed at 4°C for short-term storage or –20°C for long-term storage.

3.C. Protocol for Buccal Swabs

1. Place the head of the buccal swab into a 1.5ml microcentrifuge tube with the stick end pointing up. Add 400µl of PBS.
2. Add 20µl of Proteinase K (PK) Solution, and vortex briefly.
3. Add 400µl of Cell Lysis Buffer (CLD), and vortex for 10 seconds.
4. Incubate at 56°C for 30 minutes.
5. Remove from heat, and add 500µl of Binding Buffer (BBA). Vortex for 10 seconds.
6. Place a ReliaPrep™ Binding Column into a collection tube for each sample.
7. Add the liquid portion of the sample onto the binding column. Centrifuge in a microcentrifuge for 1 minute at maximum speed. Discard the buccal swab.
8. Place the column into a fresh collection tube. Add 500µl of Column Wash Solution (CWD) to the column, and centrifuge at maximum speed for 2 minutes. If the sample is still visible on top of the membrane, centrifuge the column for another minute.
9. Place the column in a fresh collection tube. Repeat Step 8 twice for a total of three washes.
10. Place the column into a clean, labeled 1.5ml microcentrifuge tube. Add 50–200µl of Nuclease-Free Water, and centrifuge the column for 1 minute at maximum speed.

    Note: The lower the volume of eluant added, the higher the final DNA concentration will be. However, some DNA (15–20%) will be lost at the lower volumes.
11. Discard the column. The genomic DNA can be placed at 4°C for short-term storage or –20°C for long-term storage.

4. 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**

Symptoms	Possible Causes and Comments
Wash Buffer did not pass through the column	Samples were not centrifuged long enough. Centrifuge for another 1 minute.
DNA yield is low	Samples were old. Use fresh samples, or increase the amount of sample.
	Samples were not adequately homogenized. Optimize homogenization procedure.
	Lysis was incomplete. Increase lysis time, and be sure to vortex the samples as described. The use of a shaker/heater will greatly increase lysis of solid tissue.
	Binding solution was not mixed properly with lysate. Make sure to vortex the solution for at least 10 seconds after adding the binding buffer to the lysate.
Mouse tail A_{260}/A_{230} ratios vary	DNA purified from mouse tails may show variability in A_{260}/A_{230} ratios. We have observed ratios higher than 2.2 with some batches of tails.

[a]U.S. Pat. No. 7,264,927, European Pat. No. 1442045, Japanese Pat. No. 4277115 and other patents pending.