Technical Bulletin

MagaZorb®
DNA Mini-Prep Kit

INSTRUCTIONS FOR USE OF PRODUCTS MB1004 AND MB1008.

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MagaZorb® DNA Mini-Prep Kit

Please contact Promega Technical Services if you have questions on use of this system.
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1. Description

The MagaZorb® DNA Mini-Prep Kit[a] provides an easy, fast, cost-effective method for isolating PCR-quality DNA. Using one simple protocol, a high yield of purified DNA can be isolated from a variety of sources including whole blood (fresh or frozen, citrate, heparin, or EDTA treated), buffy coat, leukocytes, milk, seminal fluid, dried blood spots, cultured cells, tissue (fresh, frozen, or formalin-fixed paraffin-embedded), saliva, urine, stool, hair, buccal swabs, and vaginal swabs.

The MagaZorb® DNA Mini-Prep Kit contains all needed reagents so that no reagent preparation is required, and does not require an organic solvent, eliminating the need for special storage or waste disposal. MagaZorb® technology enables increased sample throughput and improved reproducibility by eliminating the need for centrifugation, vacuum filtration, or column separation. DNA purified by the MagaZorb® Kit is ready for downstream applications such as PCR, sequencing or blotting procedures.

The MagaZorb® Kit is based on the specific interaction between nucleic acids and proprietary magnetizable particles in the presence of specially formulated buffer reagents. This simple, elegant technology is designed so that binding of nucleic acids is not dependent on chaotropic agents.

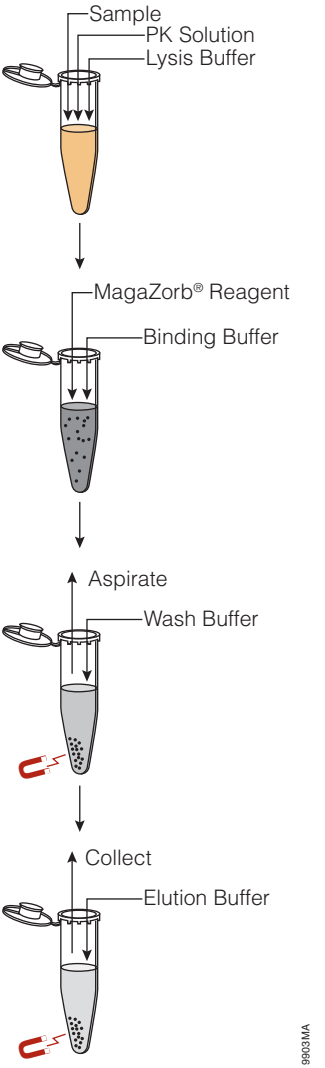

Figure 1. Schematic of the MagaZorb® DNA Mini-Prep Kit Protocol.

DNA in the sample is liberated using Proteinase K (PK) and a lysis buffer. Released DNA is bound exclusively and specifically to the MagaZorb® Reagent in presence of a binding buffer. The DNA bound to MagaZorb® particles is captured by a magnet, and contaminants are removed by washing with a single wash buffer. The DNA is then eluted from the particles with an elution buffer or molecular-grade water. A schematic representation of the MagaZorb® DNA Mini-Prep Kit protocol is shown in Figure 1.

2. Product Components and Storage Conditions

Product	Size	Cat. #
MagaZorb® DNA Mini-Prep Kit	200 preps	MB1004

Each system contains sufficient reagents for 200 purifications. Includes:

- 4ml Proteinase K (PK) Solution
- 40ml Lysis Buffer
- 100ml Binding Buffer
- 4ml MagaZorb® Reagent
- 2 × 200ml Wash Buffer
- 120ml Elution Buffer

Product	Size	Cat. #
MagaZorb® DNA Mini-Prep Kit	800 preps	MB1008

Each system contains sufficient reagents for 800 purifications. Includes:

- 16ml Proteinase K (PK) Solution
- 160ml Lysis Buffer
- 400ml Binding Buffer
- 16ml MagaZorb® Reagent
- 4 × 400ml Wash Buffer
- 480ml Elution Buffer

Storage Conditions: Store the MagaZorb® DNA Mini-Prep Kit at room temperature (15–25°C). When stored properly, all components are stable until the expiration date stated on the kit.

3. Protocols

3.A. Isolation of DNA from Blood, Buffy Coat, Leukocytes, Milk and Seminal Fluid

Materials to be Supplied By the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- end-over-end tube rotator (optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (optional)

Notes:

1. We recommend using clean, disinfected tubes and pipette tips designed for molecular biology.
2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.
3. Sample volumes can be changed to obtain the desired yields.
4. If sufficient DNA is not obtained from 200µl milk or seminal fluid samples, increase the sample volume to 500µl or 1ml. Increase the volumes of the other reagents proportionally.

Lysis

Before beginning the lysis steps:

- Bring the samples to room temperature.
- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix the solution by hand swirling. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix the buffer by hand swirling. Avoid generating bubbles or foam. If a precipitate is present, warm the buffer at 56°C for 5–10 minutes or until a clear solution is obtained.
- Immediately before adding the sample, mix the sample by pulse-vortexing for 10 seconds, or until all the precipitated cells are well dispersed.

1. Add 20µl of well-mixed PK Solution to the very bottom of a clean 2ml microcentrifuge tube. Add the PK Solution to the center of the tube, and avoid touching the sides of the microcentrifuge tube while pipetting.
2. Add 200µl of well-mixed sample into the microcentrifuge tube, directly on top of the PK Solution. Avoid touching the sides of the microcentrifuge tube while pipetting.
3. Mix by gently swirling the tube. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
4. If an RNA-free genomic DNA preparation is required, add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube. Close the tube and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.

   If an RNA-free genomic DNA preparation is not required, proceed to Step 5.
5. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.
6. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the tube cap.
7. Incubate in a 56°C water bath for 10 minutes using a foam float. Alternatively, a tight-fitting heating block at 56°C can be used.
8. Remove the tube from 56°C and dry the outside of the tube with a paper towel. If the sample used was whole blood, an olive or green color is indicative of complete lysis.

Binding

Before beginning the binding steps:

- Immediately before adding the Binding Buffer, mix the buffer by inverting or pulse-vortexing.
- Immediately before adding the MagaZorb® Reagent, mix the reagent by vortexing or rigorous hand swirling until the particles are uniformly suspended. If multiple samples are being used, the MagaZorb® Reagent should be mixed between each addition.

9. Add 500µl of the well-mixed Binding Buffer to the sample from Step 8.
10. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. This can also be achieved by simply inverting the tube 8-10 times. Tap the tube gently to release any solution caught in the tube cap.

3.A. Isolation of DNA from Blood, Buffy Coat, Leukocytes, Milk and Seminal Fluid (continued)

11. Add 20µl of well-mixed MagaZorb® Reagent directly into the solution.
12. Mix by gentle pulse-vortexing, usually 15 seconds.
13. Incubate at room temperature for 10 minutes while mixing, using an end-over-end tube rotator or occasional manual mixing (every 2 minutes). If a rotator is used, visually ensure that complete mixing is occurring.
14. Sediment the MagaZorb® particles using a magnetic rack. Sedimentation duration depends on the strength of the magnet. Particles usually sediment within 60–90 seconds. While holding the tubes onto the magnet, invert the magnet 2–3 times to "rinse" the tube cap with the supernatant.
15. Remove the supernatant by aspiration. The tube should be tightly pressed against the magnet during the aspiration to ensure the magnetic particles in the tube are tightly attached to the magnet. Remove as much liquid as possible, including any liquid trapped in the tube cap, taking care not to remove any particles.

Wash

Before beginning the washing step, mix the Wash Buffer by inverting or swirling.

16. Add 1ml of well-mixed Wash Buffer to the tube from Step 15.
17. Remove the tube from the magnet and mix well by inverting the tube several times to ensure the particles are completely dispersed.
18. Sediment the particles on the magnetic rack as in Step 14.
19. Remove the supernatant by aspiration as in Step 14.
20. Repeat Steps 16 to 20 to perform a second wash. The supernatant after the second wash should be very clear. Make sure to remove all liquid by aspiration after the second wash, including any liquid trapped in the tube cap. Take care not to remove any particles. The microcentrifuge tube should be dry, with only the MagaZorb® particles firmly pelleted at the bottom of the tube.

Elution

Before beginning the elution step:

- Immediately before adding the Elution Buffer, mix the Buffer by inverting or pulse-vortexing.

21. Add 200µl, or desired amount, of the well-mixed Elution Buffer, or molecular-grade water, to the tube from Step 20.

    Note: The standard elution volume for most applications is 200µl. Smaller elution volumes (50µl) or heated elutions (56–65°C) can also be used. For smaller elution volumes, efficient mixing of particles is critical to obtain the maximum yield of DNA. Manual mixing is recommended for elution volumes of <100µl.

22. Mix gently by inverting and swirling, after removing from the magnet.

23. Incubate the tube for 10 minutes at room temperature using an end-over-end tube rotator or occasional manual mixing (every 2 minutes).

24. Sediment the particles on the magnetic rack as in Step 14.

25. Carefully transfer the supernatant into a clean tube. Avoid collecting particles during the transfer. The transferred supernatant contains the purified DNA.

The isolated DNA is ready for further analysis. If the sample is not going to be tested on the same day, store it at –20°C until the time of analysis.

3.B. Isolation of DNA from Dried Blood Spots

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- TE Buffer: 10mM Tris-HCl, 1mM EDTA (pH 8.0)
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications.

2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.

3. This protocol has been established using the S&S 903 Paper (Schleicher & Schuell) and 25µl of blood sample per spot (~9mm diameter).

3.B. Isolation of DNA from Dried Blood Spots (continued)

Lysis

Before beginning the Lysis step:

- Bring the samples to room temperature, if applicable.
- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.

1. Cut the dried blood spot circle into 4 small pieces.
2. Add the paper pieces to a clean 2ml microcentrifuge tube.
3. Add 300µl of TE buffer to the tube.
4. Incubate the paper pieces for 30 minutes at room temperature, mixing occasionally (once every 5–10 minutes) by manual swirling.
5. Add 20µl of well-mixed PK Solution to the bottom center of the tube from Step 4. Avoid touching the sides of the tube while pipetting.
6. Mix by pulse-vortexing for 15 seconds. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
7. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 7. If an RNA-free genomic DNA preparation is not required, proceed to Step 8.

   Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 6. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.
8. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.
9. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.
10. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.
11. Remove the tube from 56°C and dry the outside with a paper towel.
12. Remove and discard the paper pieces using a pipet tip. Alternatively, the liquid can be removed from the tube by pipet and transferred into a clean 2ml microcentrifuge tube and the original tube can be discarded.

Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 100µl.

3.C. Isolation of DNA from Cultured Cells

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- 10mM PBS (pH 7.4)
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications.
2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.

Lysis

Before beginning the Lysis step:

- Bring the samples to room temperature.
- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.
- Mix the sample immediately before addition by pulse-vortexing for 10 seconds, or until all the precipitated cells are well dispersed.

1. In a clean 2ml microcentrifuge tube, resuspend the bacterial cells (2.5×10^7 cells maximum) in 200µl of PBS buffer.
2. Add 20µl of well-mixed PK Solution into the tube, at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.

3.C. Isolation of DNA from Cultured Cells (continued)

3. Mix gently by manual swirling. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.

4. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 4. If an RNA-free genomic DNA preparation is not required, proceed to Step 5.

   Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 3. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.

5. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.

6. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.

7. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.

8. Remove the tube from 56°C and dry the outside of the tube with a paper towel.

Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 200µl.

3.D. Isolation of DNA From Fresh or Frozen Tissue

To achieve maximum yield of DNA from a tissue specimen, it is essential to mechanically disrupt the tissue prior to the DNA isolation process. A tissue grinder such as the Pellet Pestle® cordless motor tissue grinder (Kimble/Kontes), or equivalent, can efficiently disrupt the tissue and aid in rapid preparation of the sample homogenate.

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- TE Buffer: 10mM Tris-HCl, 1mM EDTA (pH 8.0)
- tissue grinder
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications. Microcentrifuge tubes with longer or sharper conical bottoms are preferred for the homogenization step.

2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.

Lysis

Before beginning the Lysis step:

- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.
- Keep the tissue sample on ice at all times during Steps 1 and 2.

1. Add 5–10mg tissue to a clean 2ml microcentrifuge tube.

2. Homogenize the tissue in 200µl TE buffer using a tissue grinder. A uniform suspension should be obtained within 5–10 minutes. Keep the tissue homogenate on ice until you proceed to Step 3.

3. Add 20µl of well-mixed PK Solution into the tube from Step 2, at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.

4. Mix gently by manual swirling. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.

5. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 5. If an RNA-free genomic DNA preparation is not required, proceed to Step 6.

   Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 4. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.

6. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.

7. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.

3.D. Isolation of DNA From Fresh or Frozen Tissue (continued)

8. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.

9. Remove the tube from 56°C and dry the outside of the tube with a paper towel.

Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 200µl.

3.E. Isolation of DNA from Formalin-Fixed, Paraffin-Embedded Tissue

To achieve maximum yield of DNA from a tissue specimen, it is essential to mechanically disrupt the tissue prior to the DNA isolation process. A tissue grinder such as the Pellet Pestle® cordless motor tissue grinder (Kimble/Kontes), or equivalent, can efficiently disrupt the tissue and aid in rapid preparation of the sample homogenate.

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 75°C or tight-fitting heating block: 75°C
- microcentrifuge tubes (2ml)
- TE Buffer: 10mM Tris-HCl, 1mM EDTA (pH 8.0)
- tissue grinder
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications. Microcentrifuge tubes with longer or sharper conical bottoms are preferred for the homogenization step.

2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.

Lysis

Before beginning the Lysis step:

- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.

1. Cut a small section (10–20mg) of the formalin-fixed paraffin-embedded tissue.
2. Remove any excess of paraffin manually, as much as possible, with a spatula or other suitable tool.
3. Place the tissue in a clean 2ml microcentrifuge tube.
4. Add 1ml TE Buffer to the tube from Step 3.
5. Incubate at 75°C for 3–5 minutes.
6. Remove the tube from the water bath and gently rotate it on its side while it cools down to allow the paraffin to condense and coat the wall of the tube. This typically takes about 1 minute for 20mg of tissue.
7. Carefully transfer the tissue/TE mixture from Step 6 to a clean 2ml microcentrifuge tube, using a 1ml pipette with the pipette tip cut approximately 1cm from the tip.
8. Centrifuge the tube for 5 minutes at high speed.
9. Remove the supernatant and discard.
10. Homogenize the tissue in 200µl TE buffer using a tissue grinder. A uniform suspension should be obtained within 5–10 minutes.
11. Add 20µl of well-mixed PK Solution into the tube, at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.
12. Mix gently by manual swirling. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
13. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 13. If an RNA-free genomic DNA preparation is not required, proceed to Step 14.

    Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 12. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.

3.E. Isolation of DNA from Formalin-Fixed, Paraffin-Embedded Tissue (continued)

14. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.

15. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.

16. Incubate in a 75°C water bath for 1 hour using a foam float. A tight-fitting heating block at 75°C can also be used.

17. Remove the tube from 75°C and dry the outside of the tube with a paper towel.

    Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 200µl.

3.F. Isolation of DNA from Saliva

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications.

2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.

3. Sample volumes can be changed to obtain the desired yield. The volume of the other components should be scaled up or down accordingly.

Lysis

Before beginning the Lysis step:

- Bring the samples to room temperature.
- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.
- Mix the sample immediately before addition by pulse-vortexing for 10 seconds, or until all the precipitated cells are well dispersed.

1. Add 20µl of well-mixed PK Solution into a clean 2ml microcentrifuge tube, at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.
2. Add 200–500µl of sample into the microcentrifuge tube, directly on top of the PK Solution. Avoid touching the sides of the microcentrifuge tube while pipetting. Make sure not to pipet any bubbles.
3. Mix gently by manual swirling. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
4. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 4. If an RNA-free genomic DNA preparation is not required, proceed to Step 5.

   Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 3. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.
5. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.
6. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.
7. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.
8. Remove the tube from 56°C and dry the outside of the tube with a paper towel.

Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 100µl.

3.G. Isolation of DNA from Urine

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- TE Buffer: 10mM Tris-HCl, 1mM EDTA, pH 8.0
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications.
2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.
3. Sample volumes can be changed to obtain the desired yield. The volume of the other components should be scaled up or down accordingly.

Lysis

Before beginning the Lysis step:

- Bring the samples to room temperature.
- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.
- Mix the sample immediately before addition by pulse-vortexing for 10 seconds, or until all the precipitated cells are well dispersed.

1. Add 5–10ml urine sample into a clean centrifuge tube.
2. Centrifuge for 30 minutes at 3,500–4,000rpm.
3. Remove and discard the supernatant.
4. Add 200µl TE buffer to the tube.
5. Mix well by pulse-vortexing for 30 seconds, to dissolve the pellet.

6. Add 20µl of well-mixed PK Solution into a clean 2ml microcentrifuge tube, at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.
7. Add the mixture from Step 5 to PK Solution. Avoid touching the sides of the microcentrifuge tube while pipetting.
8. Mix gently by manual swirling. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
9. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 9. If an RNA-free genomic DNA preparation is not required, proceed to Step 10.

   Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 8. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.
10. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.
11. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.
12. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.
13. Remove the tube from 56°C and dry the outside of the tube with a paper towel.

Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 200µl.

3.H. Isolation of DNA from Stool Samples

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- 10mM PBS (pH 7.2)
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications.
2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.
3. Sample volumes can be changed to obtain the desired yield. The volume of the other components should be scaled up or down accordingly.

Lysis

Before beginning the Lysis step:

- Bring the samples to room temperature.
- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.
- Mix the sample immediately before addition by pulse-vortexing for 10 seconds, or until all the precipitated cells are well dispersed.

1. Add 5ml PBS to one gram of feces and mix to form a fecal cell suspension
2. Add 20µl of well-mixed PK Solution into a clean 2ml microcentrifuge tube, at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.
3. Add 250µl of the well-mixed fecal cell suspension from Step 1 to the bottom of the tube from Step 2.
4. Mix by pulse-vortexing for 15 seconds. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
5. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 5. If an RNA-free genomic DNA preparation is not required, proceed to Step 6.

   Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 4. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.
6. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.
7. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.
8. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.
9. Remove the tube from 56°C and dry the outside of the tube with a paper towel.

Please refer to the Binding, Wash and Elution Steps in Section 3.A.

For the Wash Step, we recommend three washes instead of the standard two washes. This will ensure complete removal of unwanted materials. The recommended elution volume for this application is 50µl.

3.I. Isolation of DNA from Hair

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications.
2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.

Lysis

Before beginning the Lysis step:

- Bring the samples to room temperature.
- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.

1. Place eight ~4cm hair strands, with roots attached, into a clean 2ml microcentrifuge tube, with the roots at the bottom of the tube.
2. Add 20µl of well-mixed PK Solution to the tube, at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.
3. Mix by pulse-vortexing for 15 seconds. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.

4. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 4. If an RNA-free genomic DNA preparation is not required, proceed to Step 5.

   Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 3. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.

5. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.

6. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.

7. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.

8. Remove the tube from 56°C and dry the outside of the tube with a paper towel. Remove the hairs with tweezers and discard

Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 100µl.

3.J. Isolation of DNA from Buccal Swabs

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- sterile cotton swabs
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications.

2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.

3.J. Isolation of DNA from Buccal Swabs (continued)

Lysis

Before beginning the Lysis step:

- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.

1. Add 200µl of well-mixed, clear Lysis Buffer to a clean microcentrifuge tube.
2. Obtain the buccal swab sample by gently rubbing the inside of the donor's cheek with a sterile cotton swab for 15–20 seconds.
3. Insert the buccal swab into the tube containing the Lysis Buffer from step 1.
4. Incubate for 2–5 minutes at room temperature with manual mixing.
5. Remove the cotton swab carefully pressing the tip against the tube wall to retain the excess liquid in the tube. Discard the swab.
6. Add 20µl of well-mixed PK Solution to the tube, at the very bottom center. Avoid touching the sides of the microcentrifuge tube while pipetting.
7. Mix by pulse-vortexing for 15 seconds. Take care not to allow any of the sample/Lysis Buffer/PK Solution to be caught in the microcentrifuge tube cap.
8. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.
9. Remove the tube from 56°C and dry the outside of the tube with a paper towel.

Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 50–70µl.

3.K. Isolation of DNA from Vaginal Swabs

We offer two procedures for isolation of DNA from vaginal swabs. The protocol described here does not involve pretreatment of the swab sample and delivers adequate yields of DNA. An alternative protocol is described in Section 3.L. The alternative protocol does involve pretreatment of the swab sample and delivers higher yields of DNA. Please choose the procedure that best fits your laboratory's needs.

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- TE Buffer: 10mM Tris-HCl, 1mM EDTA (pH 8.0)
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications.
2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.
3. Sample volumes can be changed to obtain the desired yield. The volume of the other components should be scaled up or down accordingly.

Lysis

Before beginning the Lysis step:

- Bring the samples to room temperature.
- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.
- Mix the sample immediately before addition by pulse-vortexing for 10 seconds, or until all the precipitated cells are well dispersed.

3.K. Isolation of DNA from Vaginal Swabs (continued)

1. Add 20µl of well-mixed PK Solution into the bottom of a clean 2ml microcentrifuge tube. Avoid touching the sides of the microcentrifuge tube while pipetting.
2. Add 300µl of the well-mixed swab sample (i.e., swab sample in a transportation medium, to the bottom of the tube.)
3. Mix gently by manual swirling. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
4. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 4. If an RNA-free genomic DNA preparation is not required, proceed to Step 5.

   Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 3. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.
5. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.
6. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.
7. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.
8. Remove the tube from 56°C and dry the outside of the tube with a paper towel.

Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 200µl.

3.L. Isolation of DNA from Vaginal Swabs — Alternative Protocol

We offer two procedures for isolation of DNA from vaginal swabs. The protocol described here involves pretreating the swab sample and delivers higher yields of DNA. The standard protocol is described in Section 3.K, and does not involve pretreatment of the swab sample and delivers adequate yields of DNA. Please choose the procedure that best fits your laboratory's needs.

Materials to Be Supplied by the User

- MagaZorb® Genomic DNA Mini-Prep Kit
- pipettes (20µl, 200µl, 1000µl)
- pipette tips with aerosol barrier
- vortex mixer
- magnetic rack
- water bath: 56°C or tight-fitting heating block: 56°C
- microcentrifuge tubes (2ml)
- 0.9% NaCl
- end-over-end tube rotator (Optional)
- aspiration device
- glass pipettes for aspiration
- RNase A, 20mg/ml (Optional)

Notes:

1. We recommend using clean, disinfected tubes and pipet tips specifically designed for molecular biology applications.
2. Keep tube caps closed at all times during this procedure. Only open the caps for additions, aspirations and eluate collection.
3. Sample volumes can be changed to obtain the desired yield. The volume of the other components should be scaled up or down accordingly.

Lysis

Before beginning the Lysis step:

- Bring the samples to room temperature.
- Check all buffers for precipitates.
- Immediately before adding the PK Solution, gently mix it by swirling by hand. Do not vortex.
- Immediately before adding the Lysis Buffer, gently mix it by swirling by hand. Avoid generating bubbles or foam. If a precipitate is present, warm the Buffer at 56°C for 5–10 minutes or until a clear solution is obtained.
- Mix the sample immediately before addition by pulse-vortexing for 10 seconds, or until all the precipitated cells are well dispersed.

3.L. Isolation of DNA from Vaginal Swabs — Alternative Protocol (continued)

1. Add 300µl of well-mixed swab sample (i.e., swab sample in a transportation medium), into the bottom of a clean 2ml microcentrifuge tube.
2. Centrifuge at high speed for 5–10 minutes.
3. Carefully remove and discard the supernatant with a pipette.
4. Add 500µl of 0.9% NaCl and resuspend the pellet.
5. Centrifuge at high speed for 5–10 minutes.
6. Carefully remove and discard the supernatant with a pipette.
7. Add 200µl of 0.9% NaCl to resuspend the pellet.
8. Add 20µl of well-mixed PK Solution to the tube. Avoid touching the sides of the microcentrifuge tube while pipetting.
9. Mix gently by swirling manually. Take care not to allow any of the sample/PK Solution to be caught in the microcentrifuge tube cap.
10. (Optional) If an RNA-free genomic DNA preparation is required, proceed with Step 10. If an RNA-free genomic DNA preparation is not required, proceed to Step 11.

    Add 20µl of 20mg/ml RNase A stock solution to the microcentrifuge tube from Step 9. Close the tube cap and mix gently by pulse-vortexing for 15 seconds. Incubate at room temperature for 5 minutes. Take care not to allow any of the sample/PK/RNase A mixture to be caught in the microcentrifuge tube cap.
11. Add 200µl of well-mixed, clear Lysis Buffer to the microcentrifuge tube.
12. Mix well by pulse-vortexing until a homogenous mixture is obtained, usually 15 seconds. Take care not to allow any of the sample/PK/Lysis Buffer mixture to be caught in the microcentrifuge tube cap.
13. Incubate in a 56°C water bath for 10 minutes using a foam float. A tight-fitting heating block at 56°C can also be used.
14. Remove the tube from 56°C and dry the outside of the tube with a paper towel.

Please refer to the Binding, Wash and Elution Steps in Section 3.A. The recommended elution volume for this application is 200µl.

3.M. Notes and Expected Yields

1. Typically, greater than 85% of the DNA is recovered in the first elution. If desired, more DNA can be recovered by applying a second elution.
2. A DNA control can be used for validation of the processes. When processing the control, the Lysis Step (Step 1) is not required.
3. For an RNA-free, genomic DNA preparation, 20µl of an RNase stock solution (20mg/ml) should be added to the sample before adding the Plant Lysis Buffer.
4. The DNA isolated from whole blood using the conditions described here typically has an size of ~20–30kb. DNA fragments of this length denature completely during thermal cycling and can be amplified with high efficiency.
5. Occasionally a tint of yellow color may be observed in the DNA isolated from whole blood. This will not affect the downstream processing such as PCR and sequencing.

Table 1. Expected Yields from Common Sample Types.

Sample Type	Sample Amount	Average Yield
Whole blood	200µl	5–12µg
Buffy coat (from 1.5ml of whole blood	200µl	15–50µg
Dried blood spots	25µl blood/spot	1.6–1.8µg
Hair	8 × ~4cm hairs (with root)	1.2–4.0µg
Saliva	200–500µl	1.5–4.2µg
Urine	5–10ml	3.0–8.0µg
Cerebellum tissue	5–10mg	10–30µg
Liver tissue	10mg	25–75µg
Lung tissue	5–10mg	10–25µg
Spleen tissue	5–10mg	10–40µg
Hamster blood	200µl	5.5–10.5µg
Snake blood	200µl at 1:25–1:100 dilution	100–500µg

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	Causes and Comments
Cloudy lysis buffer	Heat lysis buffer at 65°C for 5–10 minutes or until the buffer clears.
Clumping in binding or washing steps	Clumping is not usually a problem unless it is coupled with low ratio or yield. • Ensure a firm pellet is obtained when the supernatant is being removed. • Reduce sample amount/volume. • Make sure samples are completely mixed after addition of Binding Buffer. • Perform wash steps effectively.
Colored elution	Make sure samples are completely lysed.
	Make sure all the supernatant is fully aspirated during wash steps and washing is performed effectively.
Yield split among elutions	Reduce sample amount/volume.
	Increase elution volume.
Low yield	Ensure a firm pellet is obtained when the supernatant is being removed.
	Make sure samples are completely lysed.
	Avoid accidentally removing the MagaZorb® Reagent (particles) during aspiration steps.
	Allow ample time for magnetic separation.
	Increase starting sample amount.
A_{260}/A_{280} ratio is low	Ensure samples are completely lysed.
	Make sure samples are completely mixed after the Binding buffer is added.
	Make sure all the supernatant is fully aspirated during wash steps and washing is performed effectively.
A_{260}/A_{280} ratio is high	There is excessive RNA in the sample. Treat with RNase A if an RNA-free product is required.

Symptoms	Causes and Comments
When isolating DNA from Blood, there is no color change after the 56°C lysis step	Make sure both the PK and Lysis Buffer were added prior to the lysis incubation.
	Make sure that the incubation temperature was 56°C.
When isolating DNA from tissue, tissue particles do not dissolve during the lysis step	Completely homogenize the tissue at the start of the process.
	Make sure the PK and Lysis Buffer were both added prior to the lysis incubation.
	Be sure the incubation temperature was correct.

5. Related Products

For a complete list of Promega nucleic acid purification products and accessories, visit: **www.promega.com/catalog/**