MAGneat gDNA Extraction Kit
For Blood Tissue Saliva and Bacteria

2MN-BTS-96 96 Extractions

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Introduction

The MAGneat gDNA Extraction Kit provides a versatile approach to purification of high-quality DNA from a range of sample types including whole blood (collected in EDTA, Heparin or Citrate), saliva (fresh or stabilised), tissue samples and bacterial cultures. The kit utilises Microzone's proprietary buffer formulations in conjunction with MAGneat magnetic beads to reversibly and selectively bind DNA. This allows for the efficient removal of contaminants while retaining and protecting DNA. The extracted high molecular weight DNA is suitable for most downstream applications including amplification, next generation sequencing and enzymatic reactions.

Important: if automating this procedure please contact Microzone who will be able to provide guidance and support for your application.

Kit Contents

Product	Product Code	2MN-BTS-96
Lysis Buffer	2MN-LB-96	50 mL
Proteinase K	2MN-PK-96	46 mg
Binding Buffer	2MN-BB-96	15 mL
Magnetic Beads	2MN-MB-96	3.5 mL
Wash Buffer 1	2MN-WBO-96	30 mL
Elution Buffer	2MN-EB-96	28 mL

Storage and stability

Store the MAGneat Blood Tissue Saliva DNA Extraction Kit at 4°C until the expiry date displayed on the label. After reconstitution store Proteinase K at -20°C.

Magnetic Separation Devices and Plasticware

Although many brands of magnetic separation devices are compatible with the MAGneat Blood Tissue Saliva DNA Extraction Kit, we recommend the MAGneat magnetic separation range from Microzone. These products utilise neodymium magnets to ensure fast separation and defined pellets. Regardless of the magnetic separation device utilised please ensure the device is compatible with the plasticware chosen for use.

Reagent Preparation

Solvent Addition

1. Dilute the Binding Buffer with 100% Isopropanol (IPA) as described below and store at 2-8°C.

Product Code	100% IPA to be Added
2MN-BB-96	35 mL

2. Dilute the Wash Buffer with 100% ethanol as described below and store at 2-8°C.

Product Code	100% Ethanol to be Added
2MN-WBO-96	45 mL

3. Make a 75% ethanol solution with molecular grade water, 1.4 mL will be required for each reaction. This will be referred to as **Wash Buffer 2** throughout this manual. Make an excess of 10% to ensure there is sufficient to complete extractions. The table below provides dilution assistance, 10% excess is included in calculations.

Extractions	Molecular Grade Water	100% Ethanol to be Added
5	2 mL	6 mL
10	4 mL	12 mL
25	10 mL	30 mL
50	20 mL	60 mL
96	37.3 mL	112 mL

Proteinase K Reconstitution

1. Bring proteinase K vial to room temperature.

2. Remove bung and add 2.3 mL of molecular grade water to the proteinase K vial.

3. Replace bung and incubate at room temperature for 15 minutes.

4. Mix well via inversion.

5. Aliquot into required volumes and store at -20°C, avoid multiple free thaw cycles.

Note: Ensure that all solutions are at room temperature prior to use.

Whole Blood DNA Extraction Protocol

This protocol is suitable for the extraction of fresh or frozen blood samples collected in EDTA, Citrate or Heparin.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Magnetic separation device
- Vortex
- Heat block capable of 70°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Optional: RNase A (10 mg/mL)
- Optional: PBS (to make up blood samples with insufficient volume)

Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section of page 4.
- Reconstitute the Proteinase K as described in the “Proteinase K reconstitution” section on page 4.
- Set the heat block to 70°C.

Procedure

1. Prepare a mastermix of Lysis Buffer and Proteinase K, following the instructions in the below table.

Component	Amount per Prep	Total Amount per 96-well Plate
Lysis Buffer	300 µL	31.7 mL*
Proteinase K Solution	20 µL	2.1 mL*

*10% added for 96-well plate calculations.

2. Take a 2 mL centrifuge tube add 200 µL of blood and bring sample to room temperature. Add 320 µL of Lysis Buffer and Proteinase K mastermix and mix by vortexing until the sample is homogenous.

Note: If there is insufficient blood sample, make up to 200 µL with PBS.

3. Incubate samples at 70°C for 10 minutes, mixing well by vortexing after 5 minutes.

Note: After incubation, if only one heat block is in use lower the temperature to 60°C in preparation for the elution step.

Optional: Add 5 µL of RNase A (10 mg/mL), mix well by vortexing until the sample is homogenous and incubate at room temperature for 2 minutes.

4. Add 450 µL of Binding Buffer and mix well by vortexing until sample is homogenous.

5. Add 30 µL of MAGneat magnetic beads to the sample, mix well by vortexing and allow to stand at room temperature for 5 minutes. Mix after 2 minutes 30 seconds by vortexing until the sample is homogenous.

Note: Vortex the MAGneat magnetic beads thoroughly ensuring they are in solution prior to use.

6. Transfer the tube to a magnetic separation device for at least 2 minutes to magnetise the beads, then carefully remove and discard the supernatant.

7. Remove the centrifuge tube from the magnetic separation device, add 700 µL of Wash Buffer 1, mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

8. Transfer the centrifuge tube containing the homogenous sample to the magnetic separation device and magnetise for 1 minute.

9. After 1 minute, carefully remove and discard the clear supernatant.

10. Remove the centrifuge tube from the magnetic separation device, add 700 µL of Wash Buffer 2, mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

11. Transfer the tube containing the homogenous sample to a magnetic separation device and magnetise for 1 minute.

12. After 1 minute, carefully remove and discard the clear supernatant.

13. Repeat steps 10, 11 and 12 once for a total of 2 washes.

14. Remove the centrifuge tube from the magnetic separation device and let stand at room temperature for 5 to 10 minutes with the cap open to air dry the pellet.

15. During the air-drying step, fluid may accumulate at the bottom of the tube. Use a 20 µL pipette to remove any remaining supernatant if required, being careful not to disturb the pellet.

16. Add 200 µL of DNA Elution Buffer and vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall.

17. Close the cap and incubate at 60°C for 5 minutes.

18. Place the tube on a magnetic separation device and magnetise for 2 minutes or until sample is completely clear of beads.

19. Carefully transfer the clear supernatant into a new centrifuge tube for downstream processing. Be careful to not disturb the beads.

Stabilised Saliva DNA Extraction Protocol

This protocol allows the extraction of DNA from 500 µL of stabilised saliva.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Magnetic separation device
- Vortex
- Heat block capable of 70°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Optional: RNase A (10 mg/mL)
- Saliva Collection Device

Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section of page 4.
- Reconstitute the Proteinase K as described in the “Proteinase K reconstitution” section on page 4.
- Set heat block to 70°C.

Procedure

1. Add 500 µL of stabilised saliva to a 2 mL centrifuge tube.

2. Add 20 µL of Proteinase K to the tube mix well by vortexing and incubate at 70°C for 10 minutes. Mix well by vortexing after 5 minutes.

   Note: After incubation, if only one heat block is in use lower the temperature to 60°C in preparation for the elution step.

   Optional: Add 5 µL of RNase A (10 mg/mL), mix well by pipetting then incubate at room temperature for 2 minutes.

3. Add 450 µL of Binding Buffer and mix well by vortexing until sample is homogenous.

4. Add 30 µL of MAGneat magnetic beads to the sample, mix well by vortexing and allow to stand at room temperature for 5 minutes. Mix after 2 minutes 30 seconds by vortexing until the sample is homogenous.

Note: Vortex the MAGneat magnetic beads thoroughly ensuring they are in solution prior to use.

5. Transfer the tube to a magnetic separation device for at least 2 minutes to magnetise the beads, then carefully remove and discard the supernatant.

6. Remove the centrifuge tube from the magnetic separation device, add 700 µL of Wash Buffer 1, mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

7. Transfer the centrifuge tube containing the homogenous sample to the magnetic separation device and magnetise for 1 minute.

8. After 1 minute, carefully remove and discard the clear supernatant.

9. Remove the centrifuge tube from the magnetic separation device, add 700 µL of Wash Buffer 2, mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

10. Transfer the tube containing the homogenous sample to a magnetic separation device and magnetise for 1 minute.

11. After 1 minute, carefully remove and discard the clear supernatant.

12. Repeat steps 9, 10 and 11 once for a total of 2 washes.

13. Remove the centrifuge tube from the magnetic separation device and let stand at room temperature for 5 to 10 minutes with the cap open to air dry the pellet.

14. During the air-drying step, fluid may accumulate at the bottom of the tube. Use a 20 µL pipette to remove any remaining supernatant if required, being careful not to disturb the pellet.

15. Add 100 µL of DNA Elution Buffer and vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall.

16. Close the cap and incubate at 60°C for 5 minutes.

17. Place the tube on a magnetic separation device and magnetise for 2 minutes or until sample is completely clear of beads.

18. Carefully transfer the clear supernatant into a new centrifuge tube for downstream processing. Be careful to not disturb the beads.

Fresh Saliva DNA Extraction Protocol

This protocol allows the extraction of DNA from 200 µL of fresh saliva.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Magnetic separation device
- Centrifuge capable of 3000 ×g
- Vortex
- Heat block capable of 70°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Optional: RNase A (10 mg/mL)
- Suitable tube for saliva collection

Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the solvent addition section of page 4.
- Reconstitute the Proteinase K as described in the Proteinase K reconstitution section on page 4.
- Set heat block to 70°C.

Procedure

1. Generate up to 2 mL of saliva into a collection tube; do not consider the bubbles.

2. Prepare a mastermix of Lysis Buffer and Proteinase K, following the instructions in the below table.

Component	Amount per Prep	Total Amount per 96-well Plate
Lysis Buffer	300 µL	31.7 mL*
Proteinase K Solution	20 µL	2.1 mL*

*10% added for 96-well plate calculations.

3. Transfer 200 µL of saliva sample to 2 mL centrifuge tube and add 320 µL of Lysis Buffer and PK mastermix to each tube, mix well by vortexing until homogenous.

4. Incubate for 10 minutes at 70°C, mixing once after 5 minutes.

Note: After incubation, if only one heat block is in use lower the temperature to 60°C in preparation for the elution step.

5. Centrifuge the samples at 3000 ×g for 5 minutes and collect the supernatant into new 2 mL centrifuge tubes.

Optional: Add 5 µL of RNase A solution, mix well by pipetting then incubate at room temperature for 10 minutes. Mix once by vortexing after five minutes incubation.

6. Add 450 µL of Binding Buffer and mix well by vortexing until sample is homogenous.

7. Add 30 µL of MAGneat magnetic beads to the sample, mix well by vortexing and allow to stand at room temperature for 5 minutes. Mix after 2 minutes 30 seconds by vortexing until the sample is homogenous.

Note: Vortex the MAGneat magnetic beads thoroughly ensuring they are in solution prior to use.

8. Transfer the tube to a magnetic separation device for at least 2 minutes to magnetise the beads, then carefully remove and discard the supernatant.

9. Remove the centrifuge tube from the magnetic separation device, add 700 µL of Wash Buffer 1, mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

10. Transfer the centrifuge tube containing the homogenous sample to the magnetic separation device and magnetise for 1 minute.

11. After 1 minute, carefully remove and discard the clear supernatant.

12. Remove the centrifuge tube from the magnetic separation device, add 700 µL of Wash Buffer 2, mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

13. Transfer the tube containing the homogenous sample to a magnetic separation device and magnetise for 1 minute.

14. After 1 minute, carefully remove and discard the clear supernatant.

15. Repeat steps 12, 13 and 14 once for a total of 2 washes.

16. Remove the centrifuge tube from the magnetic separation device and let stand at room temperature for 5 to 10 minutes with the cap open to air dry the pellet.

17. During the air-drying step, fluid may accumulate at the bottom of the tube. Use a 20 µL pipette to remove any remaining supernatant if required, being careful not to disturb the pellet.

18. Add 100 µL of DNA Elution Buffer and vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall.

19. Close the cap and incubate at 60°C for 5 minutes.

20. Place the tube on a magnetic separation device and magnetise for 2 minutes or until sample is completely clear of beads.

21. Carefully transfer the clear supernatant into a new centrifuge tube for downstream processing. Be careful to not disturb the beads.

Homogenised Tissue DNA Extraction Protocol

This protocol allows for the extraction of DNA from up to 50 mg of tissue, using a mechanical homogeniser to process tissue samples.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Magnetic separation device
- Centrifuge capable of 13000 ×g
- Vortex
- Heat block capable of 60°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Homogeniser
- Prefilled Homogenisation Tubes
- PBS
- Optional: RNase A (10 mg/mL)

Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section of page 4.
- Reconstitute the Proteinase K as described in the “Proteinase K reconstitution” section on page 4.
- Set heat block to 60°C.

Procedure

1. Prepare a mastermix of Lysis Buffer and Proteinase K, following the instructions in the below table.

Component	Amount per Prep	Total Amount per 96-well Plate
Lysis Buffer	**500 µL**	**53.3 mL***
Proteinase K Solution	**20 µL**	**2.1 mL***

*10% added for 96-well plate calculations.

2. Weigh up to 50 mg of tissue, transfer to homogenisation tubes and add 520 µL of Lysis Buffer and Proteinase K mastermix.

3. Use homogeniser to homogenise tissue (follow the recommended protocol for your tissue type).

4. Centrifuge the homogenate at 2000 ×g until all the foam generated in the homogenisation process is removed and the sample is clear.

5. Transfer 320 µL of the homogenate to a 2 mL centrifuge tube containing 200 µL of PBS.

Optional: Add 5 µL RNase A (10 mg/mL), mix well by pipetting up/down and stand at room temperature for 10 minutes, mix once by vortexing after five minutes incubation.

6. Centrifuge sample at 13,000 ×g for 5 minutes, transfer supernatant into a new 2 mL tube, be careful to not disturb pellet.

7. Add 450 µL of Binding Buffer and mix well by vortexing until sample is homogenous.

8. Add 30 µL of MAGneat magnetic beads to the sample, mix well by vortexing and allow to stand at room temperature for 5 minutes. Mix after 2 minutes 30 seconds by vortexing until the sample is homogenous.

Note: Vortex the MAGneat magnetic beads thoroughly ensuring they are in solution prior to use.

9. Transfer the tube to a magnetic separation device for at least 2 minutes to magnetise the beads, then carefully remove and discard the supernatant.

10. Remove the centrifuge tube from the magnetic separation device and add 700 µL of Wash Buffer 1. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

11. Transfer the centrifuge tube containing the homogenous sample to the magnetic separation device and magnetise for 1 minute.

12. After 1 minute, carefully remove and discard the clear supernatant.

13. Remove the centrifuge tube from the magnetic separation device and add 700 µL of Wash Buffer 2. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

14. Transfer the tube containing the homogenous sample to a magnetic separation device and magnetise for 1 minute.

15. After 1 minute, carefully remove and discard the clear supernatant.

16. Repeat steps 13, 14 and 15 once for a total of 2 washes.

17. Remove the centrifuge tube from the magnetic separation device and let stand at room temperature for 5 to 10 minutes with the cap open to air dry the pellet.

18. During the air-drying step, fluid may accumulate at the bottom of the tube. Use a 20 µL pipette to remove any remaining supernatant if required, being careful not to disturb the pellet.

19. Add 100 µL of DNA Elution Buffer and vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall.

20. Close the cap and incubate at 60°C for 5 minutes.

21. Place the tube on a magnetic separation device and magnetise for 2 minutes or until sample is completely clear of beads.

22. Carefully transfer the clear supernatant into a new centrifuge tube for downstream processing. Be careful to not disturb the beads.

Tissue without Homogeniser Protocol

This protocol allows for the extraction of DNA from up to 50 mg of tissue.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- Magnetic separation device
- Centrifuge capable of 13000 ×g
- Vortex
- Heat block capable of 60°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Prefilled Homogenisation Tubes
- Microtube pestle
- PBS
- Optional: RNase A (10 mg/mL)

Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section of page 4.
- Reconstitute the Proteinase K as described in the “Proteinase K reconstitution” section on page 4.
- Set heat block to 60°C.

Procedure

1. Weigh up to 50 mg of tissue and transfer to a 2 mL centrifuge tube containing 500 µL of lysis buffer.

2. Using a microtube pestle grind until homogeneous.

3. Prepare a mastermix of PBS and Proteinase K, following the instructions in the below table.

Component	Amount per Prep	Total Amount per 96-well Plate
PBS	200 µL	21.1 mL*
Proteinase K Solution	20 µL	2.1 mL*

*10% added for 96-well plate calculations.

4. Transfer 300 µL of the homogenate to a 2 mL centrifuge tube containing 220 µL of the PBS and Proteinase K Mastermix, mix by vortexing until homogenous.

5. Incubate at 56°C for 1 hour, mixing every 10 minutes.

   Optional: Add 5 µL RNase A (10 mg/mL), mix well by pipetting up/down and stand at room temperature for 10 minutes, mix once by vortexing after five minutes incubation.

6. Centrifuge the samples at 13,000 ×g for 5 minutes, transfer supernatant into a new 2 mL tube, be careful to not disturb pellet.

7. Add 450 µL of Binding Buffer and mix well by vortexing until sample is homogenous.

8. Add 30 µL of MAGneat magnetic beads to the sample, mix well by vortexing and allow to stand at room temperature for 5 minutes. Mix after 2 minutes 30 seconds by vortexing until the sample is homogenous.

   Note: Vortex the MAGneat magnetic beads thoroughly ensuring they are in solution prior to use.

9. Transfer the tube to a magnetic separation device for at least 2 minutes to magnetise the beads, then carefully remove and discard the supernatant.

10. Remove the centrifuge tube from the magnetic separation device and add 700 µL of Wash Buffer 1. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

11. Transfer the centrifuge tube containing the homogenous sample to the magnetic separation device and magnetise for 1 minute.

12. After 1 minute, carefully remove and discard the clear supernatant.

13. Remove the centrifuge tube from the magnetic separation device and add 700 µL of Wash Buffer 2. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

14. Transfer the tube containing the homogenous sample to a magnetic separation device and magnetise for 1 minute.

15. After 1 minute, carefully remove and discard the clear supernatant.

16. Repeat steps 13, 14 and 15 once for a total of 2 washes.

17. Remove the centrifuge tube from the magnetic separation device and let stand at room temperature for 5 to 10 minutes with the cap open to air dry the pellet.

18. During the air-drying step, fluid may accumulate at the bottom of the tube. Use a 20 µL pipette to remove any remaining supernatant if required, being careful not to disturb the pellet.

19. Add 100 µL of DNA Elution Buffer and vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall.

20. Close the cap and incubate at 60°C for 5 minutes.

21. Place the tube on a magnetic separation device and magnetise for 2 minutes or until sample is completely clear of beads.

22. Carefully transfer the clear supernatant into a new centrifuge tube for downstream processing. Be careful to not disturb the beads.

Homogenised Bacterial DNA Extraction Protocol

This protocol allows for the extraction of DNA from bacterial culture, using a mechanical homogeniser to process bacterial samples.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- PBS
- Magnetic separation device
- Centrifuge capable of 13000 ×g
- Vortex
- Heat block capable of 60°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Homogeniser
- Prefilled Homogenisation Tubes (0.1 mm beads)
- Optional: RNase A (10 mg/mL)

Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section of page 4.
- Reconstitute the Proteinase K as described in the “Proteinase K reconstitution” section on page 4.
- Set heat block to 60°C.

Procedure

1. Prepare a mastermix of Lysis Buffer and Proteinase K, following the instructions in the below table.

Component	Amount per Prep	Total Amount per 96-well Plate
Lysis Buffer	400 µL	42.3 mL*
Proteinase K Solution	20 µL	2.1 mL*

*10% added for 96-well plate calculations.

2. Centrifuge sufficient volume of culture to provide a pellet of 50 – 100 mg wet weight or up to 10^9 bacteria.

3. Discard supernatant and resuspend the pellet in 100 µL of PBS.

4. Add 420 µL of Lysis Buffer and Proteinase K mastermix, and transfer the sample to homogenisation tubes (pre-filled tubes with 0.1 mm beads).

5. Use homogeniser to homogenise bacterial sample (suggestion: 3 cycles of 20 seconds, 30 seconds rest between cycles).

6. Centrifuge sample at 13,000 ×g for 5 minutes.

7. Transfer 320 µL of the homogenate to a 2 mL centrifuge tube containing 200 µL of PBS, be careful to not disturb pellet.

Optional: add 5 µL RNase A (10 mg/mL), mix well by pipetting up/down and stand at room temperature for 10 minutes, mix once by vortexing after five minutes incubation.

8. Add 450 µL of Binding Buffer and mix well by vortexing until sample is homogenous.

9. Add 30 µL of MAGneat magnetic beads to the sample, mix well by vortexing and allow to stand at room temperature for 5 minutes. Mix after 2 minutes 30 seconds by vortexing until the sample is homogenous.

Note: Vortex the magnetic beads thoroughly ensuring they are in solution prior to use.

10. Transfer the tube to a magnetic separation device for at least 2 minutes to magnetise the beads, then carefully remove and discard the supernatant.

11. Remove the centrifuge tube from the magnetic separation device and add 700 µL of Wash Buffer 1. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

12. Transfer the centrifuge tube containing the homogenous sample to the magnetic separation device and magnetise for 1 minute.

13. After 1 minute, carefully remove and discard the clear supernatant.

14. Remove the centrifuge tube from the magnetic separation device and add 700 µL of Wash Buffer 2. Mix sample by vortexing until homogenous, ensuring all beads have been detached from the tube wall.

15. Transfer the tube containing the homogenous sample to a magnetic separation device and magnetise for 1 minute.

16. After 1 minute, carefully remove and discard the clear supernatant.

17. Repeat steps 14, 15 and 16 once for a total of 2 washes.

18. Remove the centrifuge tube from the magnetic separation device and let stand at room temperature for 5 to 10 minutes with the cap open to air dry the pellet.

19. During the air-drying step, fluid may accumulate at the bottom of the tube. Use a 20 µL pipette to remove any remaining supernatant if required, being careful not to disturb the pellet.

20. Add 100 µL of DNA Elution Buffer and vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall.

21. Close the cap and incubate at 60°C for 5 minutes.

22. Place the tube on a magnetic separation device and magnetise for 2 minutes or until sample is completely clear of beads.

23. Carefully transfer the clear supernatant into a new centrifuge tube for downstream processing. Be careful to not disturb the beads.