MAGneat FFPE RNA Extraction Kit

2MN-FFPER-96 96 Extractions

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Contents

Introduction 2
Kit Contents 3
Storage and stability 3
Magnetic Separation Devices and Plasticware 3
Reagent Preparation 4
  Solvent Addition 4
  DNAse I Reconstitution 4
FFPE RNA Extraction Protocol 5
FFPE RNA Extraction Protocol – Automated with HeiDi-NA 11

Introduction

Formalin fixation and paraffin embedding (FFPE) is a widely used technique for preserving and archiving tissue specimens. Efficient extraction of RNA from FFPE samples enables researchers to link genetic information with tissue morphology and disease state, providing valuable insights for molecular and clinical studies.

The MAGneat FFPE RNA Extraction Kit is specifically designed for efficient purification of genomic RNA from formalin-fixed, paraffin-embedded tissue samples. The kit incorporates specialised deparaffinisation and lysis chemistry together with Microzone's proprietary buffer formulations and MAGneat magnetic beads, enabling the selective and reversible binding of RNA. This process ensures effective removal of contaminants, crosslinks, and inhibitors commonly associated with FFPE preservation, while protecting the integrity of the recovered RNA. The resulting RNA is of sufficient quality and yield for a wide range of downstream applications, including RT-PCR amplification, quantitative analysis, next generation sequencing, and other enzymatic workflows.

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-FFPER-96
FFPE RNA Deparaffinisation Solution	MN-FRDS-96	35 mL
FFPE RNA Lysis Buffer	MN-FRLB-96	31 mL
FFPE RNA Proteinase K	MN-FRPK-96	2.2 mL
FFPE RNA Binding Buffer	MN-FRBB-96	20.5 mL
FFPE RNA MAGneat Magnetic Beads	MN-FRMB-96	3 mL
FFPE RNA Wash Buffer 1	MN-FRWBO-96	28 mL
FFPE RNA DNase I (Lyophilised)	MN-FRDN-96	4400 U
FFPE RNA DNase I Reconstitution Buffer	MN-FRRB-96	480 µL
FFPE RNA DNase I Reaction Buffer	MN-FRRX-96	11 mL
FFPE RNA Elution Buffer	MN-FREB-96	21 mL

Storage and stability

All kit components can be stored at room temperature, except the MAGneat magnetic beads, which should be stored at 2–8°C to prevent microbial growth and Proteinase K, which should be stored at –20°C. After reconstitution store DNase I at -20°C.

Magnetic Separation Devices and Plasticware

Although many brands of magnetic separation devices are compatible with the MAGneat FFPE RNA 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 room temperature.

Product Code	100% IPA to be Added
MN-FRBB-96	48 mL

2. Dilute the Wash Buffer 1 with 100% ethanol as described below and store at room temperature.

Product Code	100% Ethanol to be Added
MN-FRWBO-96	42 mL

3. Make an 80% 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	1.6 mL	6.4 mL
10	3.2 mL	12.8 mL
25	8 mL	32 mL
50	16 mL	64 mL
96	30 mL	120 mL

DNAse I Reconstitution

1. Bring the DNase I tube to room temperature.
2. Remove cap and add 440 µL of DNase reconstitution buffer.
3. Replace cap and leave incubate at room temperature for 30 minutes.
4. Mix well by pipetting, do NOT vortex.
5. Aliquot into required volumes and store at -20°C, avoid multiple free thaw cycles.

FFPE RNA Extraction Protocol

This protocol is suitable for the RNA extraction of tissue samples prepared using the formalin fixation and paraffin embedding (FFPE) technique.

Materials and Reagents to be Supplied by User:

- 100% ethanol
- 100% isopropanol
- FFPE tissue blocks
- Microtome
- Magnetic separation device
- Vortex
- Heat block capable of 80°C
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water

Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section of page 4.
- Reconstitute the DNase I as described in the “DNase I reconstitution” section on page 4.
- Set the heat block to 56°C.

Procedure

<u>Preparation of FFPE Sections</u>

1. Use freshly cut FFPE tissue sections for best results.
2. Trim excess paraffin from the tissue block using a sterile blade.
3. Cut sections up to 20 µm thick from the interior of the FFPE block using a microtome.

   Note: Thicker sections may reduce nucleic acid yield.
4. Transfer the sections into a 2 mL microcentrifuge tube.
5. Gently push sections to the bottom of the tube.

   Tip: For first-time users, start with no more than three 10 µm sections to optimise results.

Deparaffinisation

6. Add 300 µL of Deparaffinisation Solution to the microcentrifuge tube containing the FFPE sections.
7. Vortex vigorously for 10 seconds, then briefly centrifuge to ensure the tissue is fully submerged.
8. Incubate at 56°C for 3 minutes.
9. Vortex to mix thoroughly.
10. Allow the sample to cool to room temperature (15–25°C).

    Note: If the sample solidifies after cooling, add additional Deparaffinisation Solution and repeat the 56 °C incubation.

Tissue Digestion & De-crosslinking

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

Component	Amount per Prep	Total Amount per 96-well Plate
FFPE Lysis Buffer	300 µL	30.24 mL*
Proteinase K Solution	20 µL	2.02 mL*

*5% added for 96-well plate calculations.

Note: Under cool ambient conditions, crystals may form in FFPE lysis buffer. Warm the bottle at 37°C to dissolve the precipitant.

12. Add 320 µL of Lysis Buffer and Proteinase K mastermix and vortex to mix thoroughly.
13. Centrifuge at 2,000 x *g* for 1 minute to create two phases within the solution: an upper oil phase and a lower aqueous phase.
14. Incubate at 56°C for 30 minutes.

Tip: Depending on sample input, tissue might need to be digested for longer period.

15. Incubate at 80°C for 15 minutes.

    Note: If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block has reached 80°C.

16. Allow the sample to cool to room temperature.

17. Centrifuge at max speed for 2 minutes to pellet debris and to collect condensation.

18. Transfer 200 µL of the lower aqueous layer to a new 2 mL microcentrifuge tube. Avoid disturbing the upper oil phase and the pelleted debris.

RNA Purification

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

Note: FFPE Binding Buffer must be diluted with 100% isopropanol prior to use. Please see instructions on Page 4.

20. Add 30 µL of MAGneat magnetic beads to the sample, mix well by vortexing and allow to stand at room temperature for 10 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.

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

22. 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.

Note: Wash Buffer 1 must be diluted with 100% ethanol prior to use. Please see instructions on Page 4.

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

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

25. 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.

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

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

28. Repeat steps 25, 26 and 27 once for a total of 2 washes.

29. 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.

30. 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.

31. Add 100 µL of RNA Elution Buffer and briefly vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall, allow to stand at room temperature for 5 minutes.

32. Prepare a mastermix of DNase I reaction buffer and the reconstituted DNase I, following the instructions in the below table. Add 5% excess to ensure you have sufficient reagent. Do **NOT** vortex!

Component	Amount per Prep
DNase I Reaction Buffer	100 µL
DNase I	4 µL

33. Add 104 µL of DNase I mastermix to each sample and mix by pipetting up/down to fully resuspend the MAGneat magnetic beads.

Note: Do **NOT** vortex samples containing DNase I.

34. Incubate samples at room temperature for 10 minutes.

35. Add 200 µL of RNA Binding Buffer, mix well by vortexing until sample is homogenous, ensuring all beads are resuspended.

36. Add 400 µL of Wash Buffer 2, mix well by vortexing.

37. Transfer the tube to a magnetic separation device for at least 1 minute or until sample is clear and all beads are magnetised. Then carefully remove and discard the supernatant.

38. Remove the centrifuge tube from the magnetic separation device, add 400 µL of Wash Buffer 2, mix sample by vortexing until sample is homogenous, ensuring all beads have been detached from the tube wall.

39. Transfer the tube to a magnetic separation device for at least 1 minute or until sample is clear and all beads are magnetised. Then carefully remove and discard the supernatant.

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

Note: do **NOT** over dry the magnetic beads as this may cause RNA degradation.

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

42. Add 100 µL of RNA Elution Buffer, vortex until the sample is homogenous, ensuring all beads have been detached from the tube wall, allow to stand at room temperature for 5 minutes.

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

44. Carefully transfer the clear supernatant into a new centrifuge tube for downstream processing being careful to not disturb the magnetic beads.

45. Store RNA at -80ºC.

FFPE RNA Extraction Protocol – Automated with HeiDi-NA

Preparation of FFPE Sections

1. Use freshly cut FFPE tissue sections for best results.
2. Trim excess paraffin from the tissue block using a sterile blade.
3. Cut sections up to 20 µm thick from the interior of the FFPE block using a microtome.

   Note: Thicker sections may reduce nucleic acid yield.
4. Transfer the sections into a 2 mL microcentrifuge tube.
5. Gently push sections to the bottom of the tube.

   Tip: For first-time users, start with no more than three 10 µm sections to optimise results.

Deparaffinisation

6. Add 300 µL of Deparaffinisation Solution to the microcentrifuge tube containing the FFPE sections.
7. Vortex vigorously for 10 seconds, then briefly centrifuge to ensure the tissue is fully submerged.
8. Incubate at 56°C for 3 minutes.
9. Vortex to mix thoroughly.
10. Allow the sample to cool to room temperature (15–25°C).

    Note: If the sample solidifies after cooling, add additional Deparaffinisation Solution and repeat the 56 °C incubation.

Tissue Digestion & De-crosslinking

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

Component	Amount per Prep	Total Amount per 96-well Plate
FFPE Lysis Buffer	300 µL	30.24 mL*
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*5% added for 96-well plate calculations.

Note: Under cool ambient conditions, crystals may form in FFPE lysis buffer. Warm the bottle at 37°C to dissolve the precipitant.

12. Add 320 µL of Lysis Buffer and Proteinase K mastermix and vortex to mix thoroughly.

13. Centrifuge at 2,000 x *g* for 1 minute to create two phases within the solution: an upper oil phase and a lower aqueous phase.

14. Incubate at 56°C for 30 minutes.

Tip: Depending on sample input, tissue might need more time to be digested.

15. Incubate at 80°C for 15 minutes.

Note: If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block has reached 80°C.

16. Allow the sample to cool to room temperature.

17. Centrifuge at max speed for 2 minutes to pellet debris and to collect condensation.

18. Transfer 200 µL of the lower aqueous layer into well number 1 of a HeiDi-NA 96 Deep Well Plate. Avoid disturbing the upper oil phase and the pelleted debris.

19. Dispense the kit's reagents into a HeiDi-NA 6 Well Sample Strips or HeiDi-NA 96 Deep Well Plates, following the table below:

	Well Number					
	1	2	3	4	5	6
Component	Binding Buffer	Magnetic Beads	Wash Buffer 1	Wash Buffer 2	Wash Buffer 2	----
Volume (mL)	450	30	700	700	700	----

20. Insert the HeiDi-NA Magnetic Tip Combs into the fixed groove of the magnetic tip comb holder in HeiDi-NA.

21. Using the QR scanner, scan the QR code to load the protocol onto the HeiDi-NA.

FFPERNA-Protocol code-1/1

22. Place the deep 96-well plate/6 well sample strip into the HeiDi-NA system and close the door.

23. Start the run.

24. Prepare a mastermix of DNase I reaction buffer and the reconstituted DNase I, following the instructions in the below table. Add 5% excess to ensure you have sufficient reagent. Do **NOT** vortex!

Component	Amount per Prep
DNase I Reaction Buffer	100 µL
DNase I	4 µL

25. Dispense the kit's reagents into a **second** HeiDi-NA 6 Well Sample Strips or HeiDi-NA 96 Deep Well Plates, following the table below:

	Well Number					
	1	2	3	4	5	6
Component	DNase I MasterMix	Wash Buffer 2	Wash Buffer 2	Elution Buffer	----	----
Volume (mL)	104	400	400	100	----	----

26. At the ‘Add Reagent’ pause (approximately 20 minutes after initial start), remove plate/strip from the instrument and replace with plate/strip #2.

    Note: It is important to replace the plates as soon as the system pauses to avoid over drying of the beads, which may be detrimental to RNA integrity.

27. With the second plate/strip into the HeiDi-NA system, close the door and press ‘Confirm’ in HeiDi's screen to continue the run.

28. At the second ‘Add Reagent’ pause (approximately 10 minutes after the previous ‘Add Reagent’ pause), remove plate/strip from the instrument.

29. Add 200 µL of Binding Buffer to the samples in wells number 1.

30. Place the deep 96-well plate/6 well sample strip back into the HeiDi-NA system, close the door and press ‘Confirm’ in HeiDi's screen to continue the run.

31. At the end of the run (approximately 50 minutes after initial start), remove the deep 96-well plate/6 well sample strip from the instrument and transfer the eluate (well 4) to a new tube or plate for downstream processing.

32. Store RNA at -80^{o}C.