MAGneat Tissue RNA Extraction Kit

2TRMN-50 50 Reactions

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Introduction

The MAGneat Tissue RNA Extraction Kit provides an effective approach to purification of high-quality RNA from tissue samples. The kit utilises Microzone's proprietary buffer formulations in conjunction with MAGneat magnetic beads to reversibly and selectively bind RNA. This allows for the efficient removal of contaminants while retaining and protecting RNA. The extracted high quality RNA 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.

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Kit Contents

Product	Product Code	2TRMN-50
Lysis Buffer	2TRLB-50	27.5 mL
Proteinase K	2TRPK-50	22 mg
Binding Buffer	2TRBB-50	10.5 mL
Magnetic Beads	2TRMN-50	1.65 mL
Wash Buffer 1	2TRWB-50	15 mL
DNase I (Lyophilised)	2TRDN-50	250 U
DNase I Reconstitution Buffer	2TRRB-50	240 µL
DNase I Reaction Buffer	2TRRX-50	5.5 mL
Elution Buffer	2TREB-50	11 mL

Storage and Stability

Store the MAGneat Tissue RNA Extraction Kit at 4°C until the expiry date displayed on the label. After reconstitution store Proteinase K and DNase I at -20°C.

Magnetic Separation Devices and Plasticware

Although many brands of magnetic separation devices are compatible with the MAGneat Tissue 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.

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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
2TRBB-50	24.5 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
2TRWB-50	22.5 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

Proteinase K Reconstitution

1. Bring Proteinase K vial to room temperature.
2. Remove bung and add 1.1 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, do **NOT** vortex.
5. Aliquot into required volumes and store at -20°C, avoid multiple freeze thaw cycles.

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DNase I Reconstitution

1. Bring the DNase I tube to room temperature.
2. Remove cap and add 220 µL of DNase reconstitution buffer.
3. Replace cap and leave incubate at room temperature for 15 minutes.
4. Mix well by pipetting, do NOT vortex.
5. Aliquot into required volumes and store at -20°C, avoid multiple free thaw cycles.

Tissue RNA Extraction Protocol

This protocol allows for the extraction of RNA 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
- Nuclease-free water
- 1X PBS
- Magnetic separation device
- Centrifuge capable of 13000 ×g
- Vortex
- Air displacement pipettes (and appropriate filter tips)
- 2 mL microcentrifuge tube compatible with the magnetic separation device
- Molecular Grade Water
- Homogeniser
- Prefilled Homogenisation Tubes

Preparation

- Prepare the Binding Buffer, Wash Buffer 1 and Wash Buffer 2 as described in the “Solvent Addition” section on page 5.

- Reconstitute the Proteinase K and DNase I as described in the "Reagent Preparation" section section on page 5 and 6.

Procedure

1. Prepare a mastermix of Lysis Buffer and Proteinase K, following the instructions in the below table. Add 5% excess to ensure you have sufficient reagent.

Component	Amount per Prep
Lysis Buffer	500 µL
Proteinase K Solution	20 µL

2. Weigh up to 50 mg of tissue, transfer to homogenisation tube 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 foam generated in the homogenisation process is removed and the sample is clear.

   Note: if tissue is not completely homogenised, consider repeat step 3 or increase the number of homogenisation cycles.

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

6. Centrifuge at 13,000 ×g for 5 minutes, transfer supernatant into a new 2 mL tube, being careful not to disturb any visible pellet formed by tissue debris.

7. Add 450 µL of RNA binding buffer and mix well by vortexing until sample is homogenous.

   Note: Sample should be mixed with binding buffer before addition of magnetic beads. Do **NOT** skip this step.

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 or until all beads are magnetised. Then carefully remove and discard the supernatant.

10. 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.

11. 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.

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 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.

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

15. Using a 20 µL pipette remove any remaining supernatant, being careful not to disturb the magnetic beads.

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 magnetic beads.

    Note: do NOT over dry as this can cause RNA degradation.

17. 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.

18. 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.

19. Prepare a mastermix of DNase I reaction buffer and 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 Solution	4 µL

20. 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.

21. Incubate samples at room temperature for 10 minutes.

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

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

24. 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.

25. 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.

26. 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.

27. Using a 20 µL pipette remove any remaining supernatant being careful not to disturb the magnetic beads.

28. 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 as this can cause RNA degradation.

29. 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.

30. 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.

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

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

33. Store RNA at -80°C.