For research use only. Not for use in diagnostic procedures.

Prepito Total RNA Kit

RNA purification from tissue samples
Product no. CMG-2035

Kit Components

Magnetic Beads	**Elution Buffer**
Lysis Buffer	**Deep Well Plates**
Binding Buffer	**0.75 mL Reaction Tubes**
Wash Buffer 3	**Disposable Tips**
Wash Buffer 4	

Completion time: approx. 45 minutes without TRIzol®/chloroform sample preparation

Typical yields:	
10 mg Brain	1 – 2 µg RNA
10 mg Kidney	7 – 9 µg RNA
10 mg Heart	2 – 3 µg RNA
10 mg Lung	7 – 8 µg RNA
10 mg Liver	7 – 9 µg RNA

Equipment and other material to be provided by the user

TRIzol® Reagent, chloroform, 2.0 mL reaction tube, 1.5 mL reaction tube, disposable gloves, pipette and pipette tips with aerosol barrier (ensure that all used material is RNase free).

Storage Conditions and Safety Information

Expiry dates are stated on the box and on each single component of the kit. Do not use any component of the kit beyond the expiration date. All kit components can be stored at room temperature.

Any further questions?
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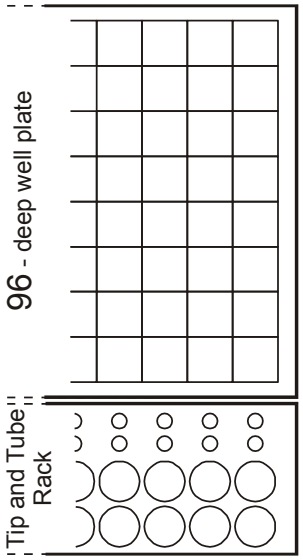
Positioning Procedure

*See "**Protocol Steps**" for detailed information*



600 µL lysate mix (300 µL Lyis Buffer / 300µL aqueous phase (upper phase) from TRIzol® based extraction of RNA from Tissue)

Pos. 4 second row for Disposable Tips; **! not used in this protocol !**
Pos. 3 Disposable Tips

Pos. 2 0.75 mL reaction tubes with 150 µL **Magnetic Beads**

Pos. 1 0.75 mL reaction tubes with 100 - 200 µL **Elution Buffer**

Before You Start

- Check all kit components for integrity. In case of damages contact your supplier.
- Connect the tubes according to their numbering to the respective counterparts at the **chemagic 8-Pack**. Remove the lids from the individual buffer bottles in the **chemagic 8-Pack** and pierce the septum with the spike placed at the end of each tube. Place the **chemagic 8-Pack** upside down on the reagent holder and use the manual priming function for a complete filling of the dispensing system.

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Protocol Steps

1. Cut a tissue piece of 10 mg and transfer it into a 2 mL tube.
2. Add 550 µL TRIzol® Reagent to the sample.
3. Disrupt the tissue with a mechanical method of your choice (Bead Mill, Rotor-Stator).
4. Centrifuge 3 minutes at 10,000 × g to precipitate insoluble debris.
5. Transfer the 500 µL supernatant into a fresh centrifuge tube (ideally with a cone shaped bottom).
6. Add 100 µL Chloroform to the solution.
7. Mix thoroughly by vortexing (at least 15 seconds).
8. Incubate at room temperature for 3 minutes.
9. Centrifuge 5 - 10 minutes at 12,000 × g at 4 °C to separate the phases.
10. Carefully transfer 300 µL of the aqueous phase (upper phase) to each well of the deep well plate (DWP, riplate SW) defined as sample well (Pos. “600 µL lysate mix (300 µL Lyis Buffer / 300µL aqueous phase (upper phase) from TRIzol® based extraction of RNA from Tissue)”, see section above “Positioning Procedure”) for subsequent extraction on the **chemagic Prepito**.
11. Switch on the **chemagic Prepito** and wait for the self test to finish.
12. Press [**Change Protocol**].
13. Press [**Tissue**] in the Select Protocol Group window.
14. Select the **Prepito Total RNA Kit** protocol by pressing [**Total RNA**] and confirm by pressing [**OK**].
15. Confirm the protocol selection in the Select Protocol Group window by pressing [**OK**].
16. Enter the 4 digit access code [**2171**] for authorization and confirm by pressing [**Enter**].
17. Press [**Start Process**].
18. Read the protocol information in the appearing information screen and confirm by pressing [**Continue**].
19. Select the sample positions and confirm by pressing [**OK**].

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20. Enter the kit barcode with the barcode scanner and confirm by pressing [**OK**].
21. For the registration of the samples and the storage tubes press [**Yes**] and follow the instructions on the touch screen panel to enter the according barcodes.
22. Prepare the **chemagic Tip & Tube Rack** with the required materials. Place one 0.75 mL reaction tube filled with 100 – 200 µL **Elution Buffer** (position 1), one 0.75 mL reaction tube filled with 150 µL **Magnetic Beads** (position 2) and one **Disposable Tip** (position 3) for each sample into the positions according to the sample positions.

! ***Shake the vessel with the Magnetic Beads vigorously until all Magnetic Beads are completely resuspended. An incomplete resuspension of the Magnetic Beads can cause a decreased yield of extracted nucleic acids.***

23. Add 300 µL Lysis Buffer to each well of the deep well plate (DWP, riplate SW) defined as sample well (Pos. “600 µL lysate mix (300 µL Lyis Buffer / 300µL aqueous phase (upper phase) from TRIzol® based extraction of RNA from Tissue)”, see section above “Positioning Procedure”) for subsequent extraction on the **chemagic Prepito**.
24. An information screen indicates the previously selected sample positions. Ensure that the sample positions in the DWP correspond to the selected positions. Place the DWP on its default position on the tracking system and press [**Continue**].
25. Place the **chemagic Tip & Tube Rack** on its default position on the tracking system. Check for accurate fit of the DWP and **chemagic Tip & Tube Rack** and lock both by closing the safety latch.
26. Close the front door and start the automated isolation process by pressing [**Start**] immediately.

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PROTOCOL

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General Remarks

The **Elution Buffer** included in this kit is 10 mM Tris-HCl pH 8.0.

The **Magnetic Bead** suspension should be mixed vigorously before dispensing, otherwise the suspension is not homogenous and the DNA yield could be low.

UV Measurements

In some cases you may find traces of **Magnetic Beads** left in the eluate. Such particles will not interfere with PCR and most downstream applications but may increase the background in UV measurements. In such a case, prior to UV analysis, we recommend an additional separation step using a manual separator (e.g. **chemagic Stand 2x12**, art. No. CMG-300) in order to separate any traces of particles.

Any further questions?

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