PROTOCOL

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

Prepito Viral NA / gDNA Kit

NA purification from 200 µl sample material

Kit Components

Magnetic Beads	**Elution Buffer**
Lysis Buffer	**Poly(A) RNA**
Binding Buffer	**Poly(A) RNA Buffer**
Wash Buffer 3	**Proteinase K**
Wash Buffer 4	**Deep Well Plates**
Wash Buffer 5	**0.75 µL Reaction Tubes**
Wash Buffer 6	**Disposable Tips**

Completion time: approximately 75 minutes

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. The kit buffers contain irritant substances. Take appropriate laboratory safety measures and wear gloves when handling.

After dissolving **Proteinase K** solution and **Poly(A) RNA** solution have to be stored at 2 – 8 °C. The solutions can be used for 6 weeks. For long term storage we recommend aliquoting the **Proteinase K** solution and the **Poly(A) RNA** solution and storing at – 20 °C.

Sample Material

The **Prepito Viral Na / gDNA Kit** can be used for different kind of body fluids such as plasma, serum, urine, liquor but also for different kind of swabs and feces suspensions. Body fluids and transport media from swabs can be used directly in aliquots of 200 µl per isolation. Feces suspensions have to be centrifuged and 200 µl of the supernatant have to be used per isolation.

Any further questions?

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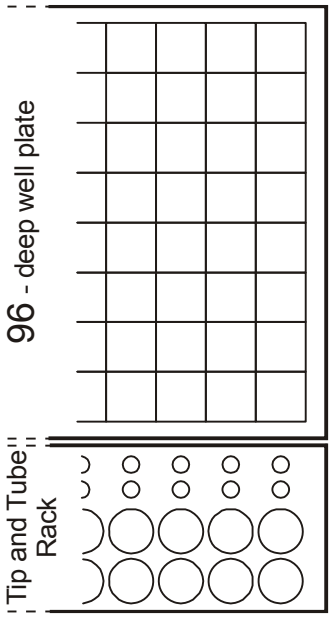
Positioning Procedure

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



200 µL sample material, Proteinase K and Poly(A)RNA

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

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

Pos. 1 0.75 µL reaction tubes with 50 - 100 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.
- Dissolve the lyophilized **Proteinase K** in RNase-free water (see instruction on the tube) and **Poly(A) RNA** in 440 µL **Poly(A) RNA Buffer** per tube.

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Protocol Steps (chemagic Prepito serial numbers 1 – 99)

1. Switch on the **chemagic Prepito** and wait for the self test to finish.
2. Press [**change protocol**].
3. Select the **Prepito Viral Na / gDNA Kit** protocol by pressing [**Viral NA + gDNA**].
4. Enter the access code **[3005]** for authorization and confirm by pressing **[enter]**.
5. Confirm the selection of the correct protocol by pressing [**enter**].
6. Read the protocol information in the appearing information screen. Confirm by pressing [**continue**].
7. Select the sample positions and confirm by pressing [**continue**].
8. Enter the kit barcode with the barcode scanner and confirm by pressing [**ok**].
9. For the registration of the samples and storage tubes press [**yes**] and follow the instructions on the touch screen panel to enter the according barcodes.
10. Prepare the **chemagic Tip & Tube Rack** with the required materials. Place one 0.75 mL reaction tube filled with 50 - 100 µL **Elution Buffer** (position 1), one 0.75 mL reaction tube filled with 150 µL of **Magnetic Beads** (position 2) and one **Disposable Tip** (position 3) for each sample into positions according to the sample positions.

! ***Shake the Magnetic Bead solution vigorously until all Magnetic Beads are completely suspended. An incomplete resuspension of the Magnetic Bead solution could cause a decreased yield of extracted nucleic acids.***

11. Add 10 µl of **Proteinase K** and 4 µl **Poly(A) RNA** to each well of the Deep Well Plate (DWP, riplate SW) defined as sample position (see section above “Positioning of Deep Well Plate and **chemagic Tip & Tube Rack**”).

! ***Don’t use Proteinase K and Poly(A) RNA for the preparation of whole blood material.***

12. Add 200 µl sample to each sample well prefilled with **Proteinase K** and **Poly(A) RNA**.
13. 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**].

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14. Place the **chemagic Tip & Tube Rack** on its default position on the tracking system. Check the accurate fit of the DWP and **chemagic Tip & Tube Rack** and lock both by closing the safety latch.

15. Close the front door and immediately start the automated isolation process by pressing [**start**].

Protocol Steps (chemagic Prepito serial numbers 100 and later)

1. Switch on the **chemagic Prepito** and wait for the self test to finish.
2. Press [**Change Protocol**].
3. Press [**Body Fluid**] in the Select Protocol Group window.
4. Select the **Prepito Viral Na / gDNA Kit** protocol by pressing [**Viral NA / gDNA**] and confirm by pressing [**OK**].
5. Confirm the protocol selection in the Select Protocol Group window by pressing [**OK**].
6. Enter the 4 digit access code [**3005**] for authorization and confirm by pressing [**Enter**].
7. Press [**Start Process**].
8. Read the protocol information in the appearing information screen and confirm by pressing [**Continue**].
9. Select the sample positions and confirm by pressing [**OK**].
10. Enter the kit barcode with the barcode scanner and confirm by pressing [**OK**].
11. 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.
12. Prepare the **chemagic Tip & Tube Rack** with the required materials. Place one 0.75 mL reaction tube filled with 50 - 100 µL **Elution Buffer** (position 1), one 0.75 mL reaction tube filled with 150 µL of **Magnetic Beads** (position 2) and one **Disposable Tip** (position 3) for each sample into positions according to the sample positions.

! ***Shake the Magnetic Bead solution vigorously until all Magnetic Beads are completely suspended. An incomplete resuspension of the Magnetic Bead solution could cause a decreased yield of extracted nucleic acids.***

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13. Add 10 µl of **Proteinase K** and 4 µl **Poly(A) RNA** to each well of the Deep Well Plate (DWP, riplate SW) defined as sample position (see section above “Positioning of Deep Well Plate and **chemagic Tip & Tube Rack**”).

! ***Don’t use Proteinase K and Poly(A) RNA for the preparation of whole blood material.***

14. Add 200 µl sample to each sample well prefilled with **Proteinase K** and **Poly(A) RNA**.
15. 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**].
16. 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.
17. Close the front door and start the automated isolation process by pressing [**Start**] immediately.

General remarks

It is strongly recommended to use the extracted nucleic acids immediately for amplification. If nucleic acid extracts cannot be used for amplification directly after preparation, the nucleic acid extracts can be kept at -20 °C or preferably at -70 °C for up to one month or one year respectively.

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

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.

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