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

Prepito Pathogen NA Extension Kit

NA purification from transport media samples (e.g.BD SurePath™).
The Kit is for use with art. No. 2020, art. No. 2021, or art. No. 2022.

Kit Components

Lysis Buffer EL 1

Lysis Buffer EL 2

Proteinase K

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 has 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 storing at - 20 °C.

Sample Material

The **Prepito Pathogen NA Extension Kit** is for the use with different types of transport media (e.g. BD SurePath™) in combination with art. No. 2020, art. No. 2021 or art. No. 2022. Depending on the used **Prepito NA Isolation Kit** different protocols have to be used. For detailed information refer to section “protocol steps”:

- Protocol [**Viral / gDNA EL**] for the use with the **Prepito Viral NA / gDNA Kit** (art. No. 2020).
- Protocol [**BF Blood EL**] for the use with the **Prepito NA Body Fluid Kit** (art. No. 2021).
- Protocol [**Pathogen NA EL**] for the use with the **Prepito Pathogen NA Kit** (art. No. 2022).

Any further questions?
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Version 120315

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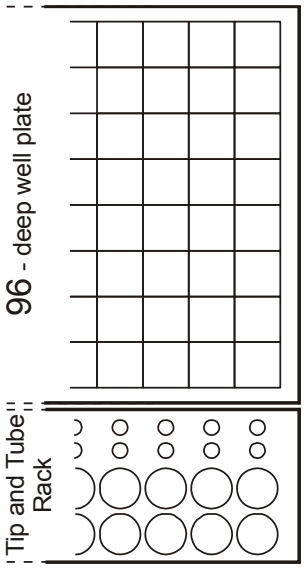
Positioning Procedure

See "Protocol Steps" for detailed information.



650 µL lysate (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 mL reaction tubes* with 150 µL **Magnetic Beads***

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

* Items provided with art. No.'s 2020, 2021 and 2022

Before You Start

- Check all kit components for integrity. In case of damages contact your supplier.
- Dissolve the lyophilized **Proteinase K** in *A. dest* (follow instructions on the tube).

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PROTOCOL

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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. Depending on the connected **Prepito NA Isolation Kit** select the [**BF Blood EL**], [**Viral / gDNA EL**] or [**Pathogen NA EL**] protocol.
4. Press [**continue**].
5. Enter your 4-digit access code [**3015**] for authorization and confirm by pressing [**enter**].
6. Confirm that the correct protocol is chosen by pressing [**enter**].
7. Read the protocol information in the appearing information screen. Confirm by pressing [**continue**].
8. Select the sample positions and confirm by pressing [**continue**].
9. Enter the kit barcode with the barcode scanner and confirm by pressing [**ok**].
10. 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.
11. Prepare the **chemagic Tip & Tube Rack** with the required materials. Place one 0.75 mL reaction tube filled with 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. **Elution Buffer**, **Disposable Tips** and **Magnetic Beads** are provided with the used **Prepito NA Isolation Kit**.

! ***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.***

12. Mix the sample or transport medium (e.g. BD SurePath™ solution) thoroughly.
13. Centrifuge 200 µL sample or transport medium for 5 minutes at 11000g.
14. Carefully discard the supernatant and resuspend in 200 µL ultra pure H_2O.
15. Add 110 µL **Lysis Buffer EL 1**, 20 µL **Proteinase K** solution and 4 µL **Poly(A)RNA** solution. **Poly(A)RNA** solution is provided with the used **Prepito NA Isolation Kit**.

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16. Incubate for 30 minutes at 56° C.
17. Add 340 µL **Lysis Buffer EL 2**.
18. Incubate for 15 minutes at 70° C.
19. Add 650 µL of the lysate to each cavity of the Deep Well Plate (DWP, riplate SW) defined as sample well (see above section “Positioning Procedure”).
20. 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**].
21. Place the **chemagic Tip & Tube Rack** to the correct position on the tracking system. Check for accurate fitting of **chemagic Tip & Tube Rack** and the DWP and lock by closing the safety latch.
22. Close the front door and immediately start the automated isolation process by pressing [**start**].

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Protocol Steps (chemagic Prepito Serial Numbers 100 - 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. Depending on the connected **Prepito NA Isolation Kit** select the [**BF Blood EL**], [**Viral NA+ gDNA EL**] or [**Pathogen NA EL**] protocol 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 [**3015**] 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 an empty 0.75 mL reaction tube filled with 100 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. Reaction tubes, **Elution Buffer**, **disposable tips** and **Magnetic Beads** are provided with the used **Prepito NA Isolation Kit**.

! ***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.***

13. Mix the sample or transport medium (e.g. BD SurePath™ solution) thoroughly.
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16. Add 110 µL **Lysis Buffer EL 1**, 20 µL **Proteinase K** solution and 4 µL **Poly(A)RNA** solution. **Poly(A)RNA** solution is provided with the used **Prepito NA Isolation Kit**.
17. Incubate for 30 minutes at 56° C.
18. Add 340 µL **Lysis Buffer EL 2**.
19. Incubate for 15 minutes at 70° C.
20. Add 650 µL of the lysate to each cavity of the Deep Well Plate (DWP, riplate SW) defined as sample well (see above section “Positioning Procedure”).
21. 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**].
22. Place the **chemagic Tip & Tube Rack** to the correct position on the tracking system. Check for accurate fitting of **chemagic Tip & Tube Rack** and the DWP and lock by closing the safety latch.
23. Close the front door and immediately start the automated isolation process by pressing **[Start]**.

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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. 300) in order to separate any traces of particles.

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