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Prepito NA Body Fluid Kit

NA purification from 200 µl sample material
Product no. CMG-2021

Kit Components

Magnetic Beads	**Wash Buffer 6**
Lysis Buffer (blood)	**Elution Buffer**
Lysis Buffer (plasma)	**Poly(A) RNA**
Binding Buffer (blood)	**Poly(A) RNA Buffer**
Binding Buffer (plasma)	**Proteinase K**
Wash Buffer 3	**Deep Well Plates**
Wash Buffer 4	**0.75 mL Reaction Tubes**
Wash Buffer 5	**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.

Lysis Buffer and **Poly(A) RNA Buffer** have to be stored in the dark. Lysis Buffer may form a precipitate upon storage. If necessary, warm to 55 °C to redissolve (after heating the **Lysis Buffer** can directly be used in the isolation process without the need of cooling down). Precipitates in the **Poly(A) RNA Buffer** can be redissolved at room temperature.

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.

Any further questions?
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Sample Material/Choice of protocol

The Kit is designed for the use with different kinds of body fluids such as whole blood (fresh or frozen),

The Kit is designed for the use with different kinds of body fluids such as whole blood (fresh or frozen), plasma, serum, urine, liquor but also for different kinds of swabs and feces suspensions. Depending on the sample material different protocols have to be used:

- Protocol [**Body Fluid - Blood**] for whole blood and sample material contaminated with whole blood. In the following protocol description these samples are named with the term "**Blood**".
- Protocol [**Body Fluid - Plasma**] for other sample material as described above. In the following protocol description these samples are named with the term "**Plasma**".

Body fluids can directly be used in aliquots of 200 µL per isolation. Transport media from swab samples can either be processed directly or the cells can be concentrated by a centrifugation step. In either case the processable volume per sample is 200 µL. Feces suspensions have to be centrifuged and 200 µL of the supernatant have to be used per isolation.

The Kit is not intended for the use with tissue as sample material. The isolation efficiency with other types of sample material has not been determined.

In some rare cases - especially with compromised blood (aged or improperly stored) - colored eluates can be observed. Colored eluates may interfere with UV measurements and may affect results in subsequent downstream applications.

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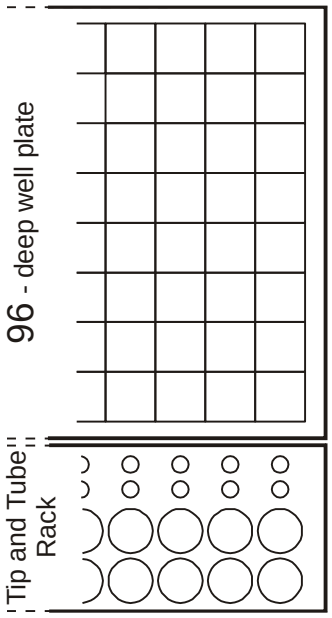
Positioning Procedure

*See "**Protocol Steps – Blood**" and "**Protocol Steps – Plasma**" for detailed information.*



200 µL sample material (opt. Proteinase K, 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 tube with 150 µL **Magnetic Beads**

Pos. 1 0.75 mL reaction tube 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 - Blood (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 NA Body Fluid Kit** protocol by pressing [**Body Fluid/Blood**].
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. For processing of sample material contaminated with blood add 4 µL **Poly(A) RNA** solution and 10 µL **Proteinase K** solution into the sample position of the Deep Well Plate (DWP; 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 material into the sample position of the Deep Well Plate (see section above “Positioning of Deep Well Plate and **chemagic Tip & Tube Rack**”).
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 - Plasma (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 NA Body Fluid Kit** protocol by pressing [**Body Fluid/Plasma**].
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 **Magnetic Beads** solution (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** solutions to each well of the Deep Well Plate (DWP) defined as sample well (DWP; see section above “Positioning of Deep Well Plate and **chemagic Tip & Tube Rack**”).

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12. Add 450 µL **Lysis Buffer (plasma)** and 200 µL sample material to each sample well prefilled with **Proteinase K** and **Poly(A) RNA** solutions.
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**].
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**].

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Protocol Steps - Blood (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 NA Body Fluid Kit** protocol by pressing [**BF Blood**] 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 **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.***

13. For processing of sample material contaminated with blood add 4 µL **Poly(A) RNA** solution and 10 µL **Proteinase K** solution into the sample position of the Deep Well Plate (DWP; 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 material into the sample position of the Deep Well Plate (see section above “Positioning of Deep Well Plate and **chemagic Tip & Tube Rack**”).

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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.

Protocol Steps - Plasma (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 NA Body Fluid Kit** protocol by pressing [**BF Plasma**] 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.

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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 **Magnetic Beads** solution (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.***

13. Add 10 µL of **Proteinase K** and 4 µL **Poly(A) RNA** solutions to each well of the Deep Well Plate (DWP) defined as sample well (DWP; see section above “Positioning of Deep Well Plate and **chemagic Tip & Tube Rack**”).
14. Add 450 µL **Lysis Buffer (plasma)** and 200 µL sample material to each sample well prefilled with **Proteinase K** and **Poly(A) RNA** solutions.
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.

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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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