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Prepito DNA Cyto Pure Kit

DNA purification from DNA from various sample materials (e.g. 250 µL blood, 10 mg tissue material and cell pellets from 1 – 5 mL amniotic fluid)

Kit Components

Magnetic Beads	**Wash Buffer 4**
Lysis Buffer B	**Elution Buffer**
Lysis Buffer T	**Proteinase K**
Binding Buffer	**Deep-Well-Plates**
Wash Buffer 1	**0.75 mL Reaction Tubes**
Wash Buffer 2	**Disposable Tips**
Wash Buffer 3	

Completion time: approx. 43 minutes (blood/tissue without lysis)
approx. 70 minutes (amniotic fluid without lysis)

Expected yields:		
	250 µL whole blood:	5 – 10 µg DNA
	10 mg liver:	24 – 40 µg DNA
	1 mL amniotic fluid:	up to 0.2 µg DNA (pregnancy week 20)

Required materials: RNAse A (100 mg/mL; not provided with the kit)

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 expiry date. All kit components can be stored at room temperature (15 – 25°C). After dissolving, **Proteinase K** solution has to be stored at 4 °C and can be used for 4 weeks. For long term storage we recommend aliquoting the **Proteinase K** solution and storing at –20 °C. Do not freeze the **Proteinase K** aliquots after thawing.

Any further questions?
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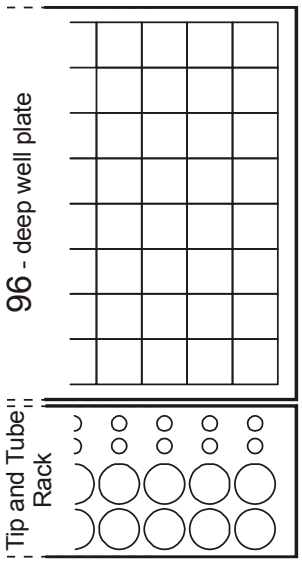
Positioning Procedure

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



sample position

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

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

Pos. 1 0.75 mL reaction tubes for **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 lyophilized **Proteinase K** in molecular biology grade water (see instruction on the tubes).
- If chorionic villi sample material is stored in tissue culture medium spin down the sample material and remove the supernatant. Wash the sample material by resuspension in 500 µL of a solution of 150 mM NaCl and 25 mM EDTA. Spin down and remove the supernatant. Ensure that the amount of sample material does not exceed 10 mg. Proceed with step 2 of the ***"Protocol Steps – Tissue samples (e.g. CVS, bone marrow, muscle etc.)***".

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Protocol Steps – Blood samples

1. Switch on the **chemagic Prepito** and wait for the self test to finish.
2. Press [**Change Protocol**].
3. Press [**Blood**] in the Select Protocol Group window.
4. Select the **Prepito DNA Cyto Pure Kit** protocol by pressing [**CP 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 [**1869**] 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 empty 0.75 mL reaction tube for the **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.***

13. Add up to 250 µL blood to each well of the Deep Well Plate (DWP, riplate SW) defined as sample well (see section above “Positioning Procdure”).

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14. Add 10 µL **Proteinase K** to each sample well prefilled with blood.

Proteinase K is required only for sample volumes more than 200 µL whole blood. Incubation of the blood/Proteinase K mixtures longer than 5 minutes can lead to lower yields and decreased purities of the extracted DNA. Therefore, continue immediately with further protocol steps after adding the Proteinase K.

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.

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Protocol Steps – Tissue samples (e.g. CVS, bone marrow, muscle etc.)

1. Transfer 10 mg of tissue sample material in small pieces (if possible) into a 2 mL micro-centrifuge tube.
2. Add 200 µL **Lysis Buffer T** and 6 µl **Proteinase K**. Incubate with agitation at 56 °C until lysis is complete. Occasionally vortexing will decrease incubation time. Lysis overnight is possible and does not influence the preparation.
3. Following lysis spin down material that is not lysed (e.g. bones, hairs) and use the supernatant for the next steps.
4. Switch on the **chemagic Prepito** and wait for the self test to finish.
5. Press [**Change Protocol**].
6. Press [**Tissue**] in the Select Protocol Group window.
7. Select the **Prepito DNA Cyto Pure Kit** protocol by pressing [**CP Tissue10**] and confirm by pressing [**OK**].
8. Confirm the protocol selection in the Select Protocol Group window by pressing [**OK**].
9. Enter the 4 digit access code [**4231**] for authorization and confirm by pressing [**Enter**].
10. Press [**Start Process**].
11. Read the protocol information in the appearing information screen and confirm by pressing [**Continue**].
12. Select the sample positions and confirm by pressing [**OK**].
13. Enter the kit barcode with the barcode scanner and confirm by pressing [**OK**].
14. 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.
15. Prepare the **chemagic Tip & Tube Rack** with the required materials. Place one empty 0.75 mL reaction tube for the **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.

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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.

16. Transfer the lysate to each well of the Deep Well Plate (DWP, riplate SW) defined as sample well (see section above “Positioning Procedure”).
17. 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**].
18. 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.
19. Close the front door and start the automated isolation process by pressing [**Start**] immediately.

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Protocol Steps – Amniotic Fluid

1. Spin down 3 – 5 mL of amniotic fluid for 15 minutes at 2050 x g to collect the cells and remove the supernatant completely.
2. Resuspend the cell pellet in 200 µL **Lysis Buffer T**.

! ***Do not resuspend the cell pellet in phosphate buffered saline (PBS).***

3. Add 6 µL **Proteinase K** solution and 8 µL **RNase A** solution (100mg/mL). Incubate for 30 minutes with agitation at 56 °C until lysis is complete. Lysis overnight is possible and does not influence the preparation.
4. Switch on the **chemagic Prepito** and wait for the self test to finish.
5. Press [**Change Protocol**].
6. Press [**Body Fluid**] in the Select Protocol Group window.
7. Select the **Prepito DNA Cyto Pure Kit** protocol by pressing [**CP Amnion**] and confirm by pressing [**OK**].
8. Confirm the protocol selection in the Select Protocol Group window by pressing [**OK**].
9. Enter the 4 digit access code [**3652**] for authorization and confirm by pressing [**Enter**].
10. Press [**Start Process**].
11. Read the protocol information in the appearing information screen and confirm by pressing [**Continue**].
12. Select the sample positions and confirm by pressing [**OK**].
13. Enter the kit barcode with the barcode scanner and confirm by pressing [**OK**].
14. 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.
15. 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 65 µL **Magnetic Beads** (position 2) and one **Disposable Tip** (position 3) for each sample into the positions according to the sample positions.

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! ***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.***

16. Transfer the lysate to each well of the Deep Well Plate (DWP, riplate SW) defined as sample well (see section above “Positioning Procedure”).
17. 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**].
18. 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.
19. Close the front door and start the automated isolation process by pressing [**Start**] immediately.

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.

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