PROTOCOL

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

Prepito DNA Cyto Pure Kit

DNA purification from Buccal Swabs

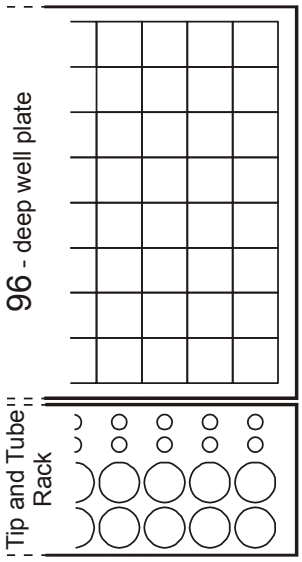
Completion time: approx. 68 minutes

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 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.

Positioning Procedure

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



400 µL Supernatant

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**

Any further questions?
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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 – Buccal Swabs

1. A premix of Proteinase K and **Lysis Buffer T** (10µl Proteinase K and 500 µl **Lysis Buffer T** /swab) can be dispensed in an appropriate reaction tube (e.g. 1.5 ml reaction tube). Place the swabs in the tubes prefilled with buffer and incubate for 10 min at 56°C.

! ***The Proteinase K activity will decrease after incubation longer than 10 minutes in*** **Lysis Buffer T*****. Ensure that all swabs are placed in the Proteinase K/*** **Lysis Buffer T** ***within this time.***

2. Following lysis remove the swab and spin down material that is not lysed (swab material) and use 400 µl supernatant for the next steps.
3. Switch on the **chemagic Prepito** and wait for the self test to finish.
4. Press [**Change Protocol**].
5. Press [**Body Fluid**] in the Select Protocol Group window.
6. Select the **Prepito DNA Cyto Pure Kit** protocol by pressing [**CP Buccal-S**] and confirm by pressing [**OK**].
7. Confirm the protocol selection in the Select Protocol Group window by pressing [**OK**].
8. Enter the 4 digit access code [**2121**] for authorization and confirm by pressing [**Enter**].
9. Press [**Start Process**].
10. Read the protocol information in the appearing information screen and confirm by pressing [**Continue**].
11. Select the sample positions and confirm by pressing [**OK**].
12. Enter the kit barcode with the barcode scanner and confirm by pressing [**OK**].
13. 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.
14. 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 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.***

15. Transfer 400 µL supernatant to each well of the Deep Well Plate (DWP, riplate SW) defined as sample well (see section above “Positioning Procdure”).
16. 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**].
17. 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.
18. 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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