PROTOCOL

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Prepito FFPE Kit

DNA purification from 10 µm sections or equivalent of FFPE tissue
Product no. CMG-2027

Kit Components

Magnetic Beads	**Wash Buffer 6**
Lysis Buffer	**Elution Buffer**
Binding Buffer	**Proteinase K**
Wash Buffer 3	**Deep Well Plates**
Wash Buffer 4	**0.75 mL Reaction Tubes**
Wash Buffer 5	**Disposable Tips**

Completion time: approx. 45 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.

After dissolving **Proteinase K** solution has to be stored at 2 – 8 °C. The solution 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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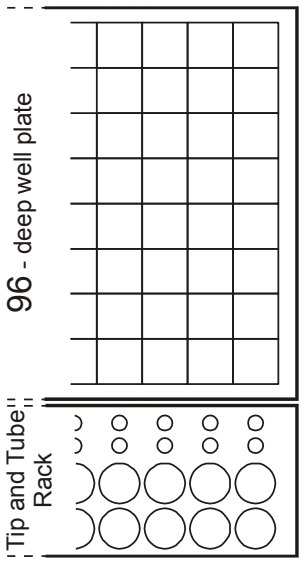
Positioning Procedure

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



200 µL lysate

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 for 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 Aqua dest. (see instruction on the tube).

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Protocol Steps / Xylene (chemagic Prepito serial numbers 1 – 99)

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut sections 10 – 20 µm thick.
3. If the sample surface has been exposed to air, discard the first 2 – 3 sections.
4. Transfer the cuts into a 2.0 mL reaction tube.
5. Do not use more than 5 mg total amount of FFPE tissue material (approximately four 10 µm sections of 150 mm^2 surface area or two 20 µm sections of 150 mm^2 surface area).

! ***The total yield of DNA will strictly depend on the amount of starting material. The total amount of tissue should not exceed 5 mg.***

6. Add 1 mL Xylene to the paraffin-embedded tissue. Vortex vigorously.
7. Incubate 10 min at room temperature.
8. Centrifuge at full speed for 5 min.
9. Remove supernatant by carefully pipetting.
10. Add 1 mL Ethanol (96 – 100 %), vortex and incubate 10 min room temperature.
11. Centrifuge at full speed for 5 min.
12. Remove supernatant by carefully pipetting.
13. Repeat Ethanol washing (step 11/step12).
14. Incubate the tube with the lid open at 37°C until the Ethanol has evaporated (10 – 15 min).
15. Add 200 µL **Lysis Buffer** 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.
16. Following lysis spin down material that is not lysed and use the supernatant for the next steps.
17. Switch the **chemagic Prepito** on and wait until the self test is finished.
18. Press [**change protocol**].
19. Select the **Prepito FFPE Kit** protocol by pressing [**FFPE**].
20. Enter the access code [**4895**] for authorization and confirm by pressing [**enter**].

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21. Confirm the selection of the correct protocol by pressing [**enter**].
22. Read the protocol information in the appearing information screen. Confirm by pressing [**continue**].
23. Select the sample positions and confirm by pressing [**continue**].
24. Enter the kit barcode with the barcode scanner and confirm by pressing [**ok**].
25. 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.
26. 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 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.***

27. Transfer the lysate to each well of the Deep Well Plate (DWP, riplate SW) defined as sample well (Pos. “200 µL tissue lysate”, see section above “Positioning Procedure”).
28. 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**].
29. 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.
30. Close the front door and immediately start the automated isolation process by pressing [start] immediately.

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Protocol Steps / Xylene (chemagic Prepito serial numbers 100 and later)

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut sections 10 – 20 µm thick.
3. If the sample surface has been exposed to air, discard the first 2 – 3 sections.
4. Transfer the cuts into a 2.0 mL reaction tube.
5. Do not use more than 5 mg total amount of FFPE tissue material (approximately four 10 µm sections of 150 mm^2 surface area or two 20 µm sections of 150 mm^2 surface area).

! ***The total yield of DNA will strictly depend on the amount of starting material. The total amount of tissue should not exceed 5 mg.***

6. Add 1 mL Xylene to the paraffin-embedded tissue. Vortex vigorously.
7. Incubate 10 min at room temperature.
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9. Remove supernatant by carefully pipetting.
10. Add 1 mL Ethanol (96 – 100 %), vortex and incubate 10 min room temperature.
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13. Repeat Ethanol washing (step 11/step12).
14. Incubate the tube with the lid open at 37 °C until the Ethanol has evaporated (10 – 15 min).
15. Add 200 µL **Lysis Buffer** 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.
16. Following lysis spin down material that is not lysed and use the supernatant for the next steps.
17. Switch on the **chemagic Prepito** and wait for the self test to finish.
18. Press [**Change Protocol**].
19. Press [**Tissue**] in the Select Protocol Group window.
20. Select the **Prepito FFPE Kit** protocol by pressing [**FFPE**] and confirm by pressing [**OK**].

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21. Confirm the protocol selection in the Select Protocol Group window by pressing [**OK**].
22. Enter the 4 digit access code [**4895**] for authorization and confirm by pressing [**Enter**].
23. Press [**Start Process**].
24. Read the protocol information in the appearing information screen and confirm by pressing [**Continue**].
25. Select the sample positions and confirm by pressing [**OK**].
26. Enter the kit barcode with the barcode scanner and confirm by pressing [**OK**].
27. 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.
28. 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 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.***

29. Transfer the lysate to each well of the Deep Well Plate (DWP, riplate SW) defined as sample well (Pos. “200 µL tissue lysate”, see section above “Positioning Procedure”).
30. 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**].
31. 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.
32. Close the front door and start the automated isolation process by pressing [**Start**] immediately.

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Protocol Steps / Organic Solvent free (chemagic Prepito serial numbers 1 – 99)

1. Transfer the paraffin-embedded tissue sample (up to three 10 µm sections) into a 1.5 mL microcentrifuge tube.
2. Add 180 µL Lysis Buffer 1, close the tube and heat up to 99 °C for 15 minutes in order to melt the paraffin. After 5 minutes finger-flip the tubes to mix the content.
3. Cool down at 65 °C and add 35 µL Proteinase K.
4. Incubate at 65 °C for 15 minutes with slight agitation

! ***The 15 minutes described is set as a fast lysis procedure with focus on time saving, not on maximal yield. Yield is increasing if digestion time is prolonged up to 4h***

5. Centrifuge at full speed for 5 min.
6. Switch the **chemagic Prepito** on and wait until the self test is finished.
7. Press [**change protocol**].
8. Select the **Prepito FFPE Kit** protocol by pressing [**FFPE**].
9. Enter the access code [**4895**] for authorization and confirm by pressing [**enter**].
10. Confirm the selection of the correct protocol by pressing [**enter**].
11. Read the protocol information in the appearing information screen. Confirm by pressing [**continue**].
12. Select the sample positions and confirm by pressing [**continue**].
13. Enter the kit barcode with the barcode scanner and confirm by pressing [**ok**].
14. 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.
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 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 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.***

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16. Transfer the lysate to each well of the Deep Well Plate (DWP, riplate SW) defined as sample well (Pos. “200 µL tissue lysate”, 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 immediately start the automated isolation process by pressing [start] immediately.

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Protocol Steps / Organic Solvent free (chemagic Prepito serial numbers 100 and later)

1. Transfer the paraffin-embedded tissue sample (up to three 10 µm sections) into a 1.5 mL microcentrifuge tube.
2. Add 180 µL Lysis Buffer 1, close the tube and heat up to 99 °C for 15 minutes in order to melt the paraffin. After 5 minutes finger-flip the tubes to mix the content.
3. Cool down at 65 °C and add 35 µL Proteinase K.
4. Incubate at 65 °C for 15 minutes with slight agitation

! ***The 15 minutes described is set as a fast lysis procedure with focus on time saving, not on maximal yield. Yield is increasing if digestion time is prolonged up to 4h***

5. Centrifuge at full speed for 5 min.
6. Switch on the **chemagic Prepito** and wait for the self test to finish.
7. Press [**Change Protocol**].
8. Press [**Tissue**] in the Select Protocol Group window.
9. Select the **Prepito FFPE Kit** protocol by pressing [**FFPE**] and confirm by pressing [**OK**].
10. Confirm the protocol selection in the Select Protocol Group window by pressing [**OK**].
11. Enter the 4 digit access code [**4895**] for authorization and confirm by pressing [**Enter**].
12. Press [**Start Process**].
13. Read the protocol information in the appearing information screen and confirm by pressing [**Continue**].
14. Select the sample positions and confirm by pressing [**OK**].
15. Enter the kit barcode with the barcode scanner and confirm by pressing [**OK**].
16. 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.
17. 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

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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 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.***

18. Transfer the lysate to each well of the Deep Well Plate (DWP, riplate SW) defined as sample well (Pos. “200 µL tissue lysate”, see section above “Positioning Procedure”).
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20. 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.
21. 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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