Paraffin embedded tissue kit

For research use only

Content	DN1211
Buffer DATL	14 ml
Buffer DAL	12 ml
Buffer DAW1	19 ml
Buffer DAW2	13 ml
Buffer DATE	20 ml
Proteinase K	1.25 ml
Spin Column	50

Store at: RT

Store proteinase K at: 2-8 °C

Intended Use

The Paraffin embedded tissue kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease. All due care and attention should be exercised in the handling of the products.

Introduction

The Paraffin embedded tissue kit is optimized for purification of DNA from Paraffin embedded tissue sections. It uses well-established DNA Micro technology for purification of genomic and mitochondrial DNA from small sample volumes or sizes. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes of between 20 and 1 00 µl. specially optimized lysis conditions allow genomic DNA to be efficiently purified from Paraffin embedded tissue sections without the need for overnight incubation. Incubation at an elevated temperature after proteinase K digestion partially removes formalin crosslinking of the released DNA, improving yields, as well as DNA performance in downstream assays.

Note that DNA isolated from Paraffin embedded samples is usually of lower molecular weight than DNA from fresh or frozen samples. The degree of fragmentation depends on the type and age of the sample and the conditions used for fixation. After sample lysis, the simple DNA Micro procedure, which is highly suited for simultaneous processing of multiple samples, yields pure DNA in less than 30 minutes. DNA is eluted in Buffer DATE or water and is immediately ready for use in amplification reactions or for storage at –20°C. Purified DNA is free of proteins, nucleases, and other impurities.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- **Xylene**
- **Ethanol (96–1 00%)***
- **1 .5 ml or 2 ml microcentrifuge tubes (for lysis steps)**
- **1 .5 ml microcentrifuge tubes (for elution steps) (available from Brinkmann, Eppendorf or Sarstedt**
- **Pipet tips (to avoid cross-contamination, we recommend pipet tips with aerosol barriers)**
- **Thermomixer, heated orbital incubator, heating block, or water bath capable of incubation at 90°C**
- **Microcentrifuge with rotor for 2 ml tubes**
- **Vortexer**
- **Optional: RNase A**

Starting material

Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation of nucleic acids. To limit the extent of DNA fragmentation, be sure to:

- **Fix tissue samples in 4–1 0% formalin as quickly as possible after surgical removal.**
- **Use a fixation time of 1 4–24 hours (longer fixation times lead to more severe DNA fragmentation, resulting in poor performance in downstream assays).**
- **Thoroughly dehydrate samples prior to embedding (residual formalin can inhibit the proteinase K digest).**

Starting material for DNA purification should be freshly cut sections of Paraffin embedded tissue, each with a thickness of up to 1 0 µm. Up to 8 sections, each with a thickness of up to 1 0 µm and a surface area of up to 250 mm2, can be combined in one preparation. If you have no information about the nature of your starting material, we recommend starting with no more than 3 sections per preparation. Depending on DNA yield and purity, it may be possible to use up to 8 sections in subsequent preparations.

Handling of Spin columns

Due to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling Spin columns to avoid cross contamination between sample preparations:

- **Carefully apply the sample or solution to the column. Pipet the sample into the Spin column without wetting the rim of the column.**
- **Always change pipet tips between liquid transfers. We recommend the use of aerosol-barrier pipet tips.**
- **Avoid touching the Spin column membrane with the pipet tip.**
- **After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to**

remove drops from the inside of the lids.
■ Open only one Spin column at a time, and take care to avoid generating aerosols.
■ Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Centrifugation

Spin columns will fit into most standard 1 .5–2 ml microcentrifuge tubes.
Additional 2 ml collection tubes are available separately.
Centrifugation of Spin columns is performed at 6000 x g (8000 rpm) to reduce centrifuge noise. Centrifugation at full speed will not improve DNA yields. However, centrifugation of Spin columns at full speed is required in 2 steps of the procedure: the dry centrifugation step after the membranes are washed and the elution step. Centrifugation at full speed is also required to bring down the sample after the xylene treatment and the ethanol wash step.
All centrifugation steps should be carried out at room temperature (1 5–25°C).

Preparation of buffers

Preparing Buffer DATL
Before starting the procedure, check whether precipitate has formed in Buffer DATL. If necessary, dissolve by heating to 70°C with gentle agitation.

Preparing Buffer DAL
Before starting the procedure, check whether precipitate has formed in Buffer DAL. If necessary, dissolve by heating to 70°C with gentle agitation.

Preparing Buffer DAW1
Add 25 ml ethanol (96–1 00%) to the bottle containing 1 9 ml Buffer DAW1 concentrate.
Tick the check box on the bottle label to indicate that ethanol has been added.
Reconstituted Buffer DAW1 can be stored at room temperature (1 5–25°C) for up to 1 year.
Note: Before starting the procedure, mix reconstituted Buffer DAW1 by shaking.

Preparing Buffer DAW2
Add 30 ml ethanol (96–1 00%) to the bottle containing 1 3 ml Buffer AW2 concentrate.
Reconstituted Buffer AW2 can be stored at room temperature (1 5–25°C) for up to 1 year.
Note: Before starting the procedure, mix reconstituted Buffer AW2 by shaking.

Protocol:

Important points before starting
■ Perform all centrifugation steps at room temperature (1 5–25°C).
■ Read “Important Notes”, pages 9–1 1 .

Things to do before starting
■ Equilibrate all buffers to room temperature (1 5–25°C).
■ Set a the rmomixer or heated orbital incubator to 56°C for use in step 1 1 . If a the rmomixer or heated orbital incubator is not available, a heating block or water bath can be used instead.
■ If Buffer DAL or Buffer DATL contain precipitates, dissolve by heating to 70°C with gentle agitation.
■ Ensure that Buffer DAW1 and Buffer DAW2 have been prepared according to the instructions described.

Procedure

1 . Using a scalpel, trim excess paraffin off the sample block.
2. Cut up to 8 sections 5–1 0 µm thick (see “Starting material”).If the sample surface has been exposed to air, discard the first 2–3 sections.
3. Immediately place the sections in a 1 .5 or 2 ml microcentrifuge tube (not supplied), and add 1 ml xylene to the sample. Close the lid and vortex vigorously for 1 0 s.
4. Centrifuge at full speed for 2 min at room temperature (1 5–25°C).
5. Remove the supernatant by pipetting. Do not remove any of the pellet.
6. Add 1 ml ethanol (96–1 00%) to the pellet, and mix by vortexing. The ethanol extracts residual xylene from the sample.
7. Centrifuge at full speed for 2 min at room temperature.
8. Remove the supernatant by pipetting. Do not remove any of the pellet.
Carefully remove any residual ethanol using a fine pipet tip.
9. Open the tube and incubate at room temperature or up to 37°C. Incubate for 1 0 min or until all residual ethanol has evaporated.
1 0. Resuspend the pellet in 1 80 µl Buffer ATL. Add 20 µl proteinase K, and mix by vortexing.
1 1 . Incubate at 56°C for 1 h (or until the sample has been completely lysed).
1 2. Incubate at 90°C for 1 h.
The incubation at 90°C in Buffer DATL partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA. If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block has reached 90°C.
1 3. Briefly centrifuge the 1 .5 ml tube to remove drops from the inside of the lid.
If RNA-free genomic DNA is required, add 2 µl RNase A (1 00 mg/ml) and incubate for 2 min at room temperature before continuing with step 1 4. Allow the sample to cool to room temperature before adding RNase A.
1 4. Add 200 µl Buffer DAL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–1 00%), and mix again thoroughly by vortexing. It is essential that the sample, Buffer DAL, and ethanol are mixed immediately and
thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer DAL and ethanol can be premixed and added together in one step to save time when processing multiple samples. A white precipitate may form on addition of Buffer DAL and ethanol. This precipitate does not interfere with the procedure.
1 5. Briefly centrifuge the 1 .5 ml tube to remove drops from the inside of the lid.
1 6. Carefully transfer the entire lysate to the spin column (in a 2 ml collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x g
(8000 rpm) for 1 min. Place the spin column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through. If the lysate has not completely passed

through the membrane after centrifugation, centrifuge again at a higher speed until the spin column is empty.

1 7. Carefully open the spin column and add 500 µl Buffer DAW1 without wetting the rim. Close the lid and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the spin column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
1 8. Carefully open the spin column and add 500 µl Buffer DAW2 without wetting the rim. Close the lid and centrifuge at 6000 x g (8000 rpm) for 1 min.
Place the spin column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through. Contact between spin column and the flow-through should be avoided. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, coming into contact with the spin column. Take care when removing the spin column and collection tube from the rotor, so that flow-through does not come into contact with the spin column.
1 9. Centrifuge at full speed (20,000 x g; 1 4,000 rpm) for 3 min to dry the membrane completely. This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
20. Place the spin column in a clean 1 .5 ml microcentrifuge tube (not provided), and discard the collection tube containing the flow-through. Carefully open the lid of the spin column and apply 20–1 00 µl Buffer DATE to the center of the membrane.
Important: Ensure that Buffer DATE is equilibrated to room temperature. If using small elution volumes (<50 µl), dispense Buffer DATE onto the center of the membrane to ensure complete elution of bound DNA.Spin columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. The volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.
21 . Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x g; 1 4,000 rpm) for 1 min. Incubating the spin column loaded with Buffer DATE for 5 min at room
temperature before centrifugation generally increases DNA yield.

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