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QIAamp DNA FFPE Tissue Kit protocol

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

The QIAamp DNA FFPE Tissue Kit is optimized for purification of DNA from FFPE tissue sections. It uses well-established QIAamp 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 100 µl.

Specially optimized lysis conditions allow genomic DNA to be efficiently purified from FFPE 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 FFPE 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 QIAamp 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 ATE or water and is immediately ready for use in amplification reactions or for storage at –30°C to –15°C. Purified DNA is free of proteins, nucleases and other impurities.

EXTERNAL LINK

https://www.qiagen.com/cl/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-dna-ffpe-tissue-kit/?clear=true#orderinginformation

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PROTOCOL CITATION

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IMAGE ATTRIBUTION

https://www.qiagen.com/cl/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/qiaamp-dna-ffpe-tissue-kit/?clear=true#orderinginformation

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GUIDELINES

QIAamp DNA FFPE Tissue Kit should be stored at **2 °C** - **8 °C** (mandatory for columns and proteinase K and for long term storage).

All centrifugation steps should be carried at **Room temperature (15-25°C)**

MATERIALS TEXT

QIAamp DNA FFPE Tissue Kit (50) (REF 56404)

Reagents and equipment to be supplied by user:

- Xylene
- Ethanol (96–100%)*
- 15 ml conical tubes for ethanol and for xylene waste
- 1.5 ml microcentrifuge tubes (for lysis steps)
- 1.5 ml microcentrifuge tubes (for elution steps) (available from Brinkmann [SafeLock, cat. no. 022363204], Eppendorf [Safe-Lock, cat. no. 0030 120.086] or Sarstedt [Safety Cap, cat. no. 72.690])†
- 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 (100 mg/ml; cat. no. 19101) protocol)

SAFETY WARNINGS

Use PPE at all times when working with biologic samples and chemicals.
Do not add bleach or acidic solutions to the smaple preparation waste: Use detergent and water and only then 1% v/v sodium hypochlorite.

Flow-through fractions may contain hazardous waste and should be disposed appropiately.

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BEFORE STARTING

Regarding the FFPE samples:

Use a scalpel to trim the excess paraffin off the smaple block. Discard **15 µm** and cut 3 **10 µm** sections up to 250 mm^2 FFPE samples (fixed in 10% v/v neutrally buffered formalin, for 14-24 hours and properly processed) prior to the procedure. Samples should be cut no longer than 1 day before the extraction and stored at **4 °C** in a 1.5 ml tube in a clean environment.

Set the heat block at **56 °C** .

Dissolve precipitate of Buffer ATL (tissue lysis buffer) **Qiagen Catalog #19076** and Buffer AL, Lysis buffer **Qiagen Catalog #19076** at **70 °C** and gentle agitation.

Equilibrate Buffer AL, Lysis buffer **Qiagen Catalog #19076** solution at **Room temperature (15-25°C)** before applying to MinElute column. Yields increase when the column is incubated with equilibrated ATE solution for 5 minutes before centrifugation.

Add **25 mL** ethanol (96-100%) to the bottle containing **19 mL** Buffer AW1, Wash buffer (1), concentrate **Qiagen Catalog #19081** and tick the checkbox on the bottle label to indicate that ethanol has been added. Reconstituted buffer can be stores at **Room temperature** for up to 1 year. Before starting any procedure, mix by shaking.

Add **30 mL** ethanol (96-100%) to the bottle containing **13 mL** Buffer AW2, Wash buffer (2), concentrate **Qiagen Catalog #19072** and tick the checkbox on the bottle label to indicate that ethanol has been added. Reconstituted buffer can be stores at **Room temperature** for up to 1 year. Before starting any procedure, mix by shaking.

Use a **55 µl** elution volume If intended use is a Vienna strip assay.

For periods longer than 24 hours of storage, samples should be frozen (**-30 °C** - **-15 °C**).

First section preparation

1 Prepare the workspace and leavy at hand the Buffer ATL **Qiagen Catalog #19076** (see the Before start section), Proteinase K **Qiagen Catalog #19131** and ethanol **1 mL** per sample. Other reagents will be used after the first 1 hour incubation.

FFPE slices processing 1h 30m

2 Add **1 mL** Xylene to the sample, close the lid and shake vigorously for **00:00:10** 10s

3 **20000 x g, Room temperature , 00:02:30 , (full speed) (30 seconds consider the time used to get to 20,000 x g)**

and **go to step #2**

4 Remove the supernatant by pipetting. **Do not remove any of the pellet**.

5 Add **1 mL** ethanol (96-100%) to the pellet and mix by vortexing.

6 Centrifuge
20000 x g, Room temperature , 00:02:30 , (full speed) (30 seconds consider the time used to get to 20,000 x g)

7 Remove supernatant by pipetting without removing any pellet, using a **fine pipet tip**.

7.1 With the lid closed, invert the tube and flick the bottom to spread the ethanol volume across the tube.

8 Open the tube and incubate at **Room temperature** or **37 °C** for **00:10:00** or until the residual ethanol has evaporated. 10m

9 Buffer ATL
Resuspend pellet in **180 µl** of **Qiagen Catalog #19076** , add **20 µl**
Proteinase K **Qiagen Catalog #19131** and mix by vortexing.

Long incubations 2h 30m

10 1h

Incubate at **56 °C** for **01:00:00** **(or until the sample has been completely lysed)**

11 Incubate at **90 °C** for **01:00:00** 1h

Incubation at 90°C in Buffer ATL 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 sample at room temperature after the 56°C incubation until the heating block has reached 90°C.

Isolation and elution 2h 30m

12 **00:15:00** before incubation time ends, prepare and leave at hand 1.5 ml microcentrifuge tubes (1 per sample), 0.5 ml tubes (2 per sample), collection tubes (3 per sample), buffer **Qiagen Catalog #19076** , Buffer AL, Lysis 15m

Buffer
ethanol (96-100%) (**200 µl per sample**), AW1 **Qiagen Catalog #19081** ,
Buffer
AW2 **Qiagen Catalog #19072** and Buffer ATE.

13 Briefly centrifuge the 1.5 ml tube to remove drops from the lid.

14 2m

Allow the sample to cool to **Room temperature** before adding RNAse A. Add **2 µl**
RNase
A **Qiagen Catalog #19101** and incubate for **00:02:00** at **Room temperature** before continuing with step 15.

15 Buffer AL, Lysis
Add **200 µl** of buffer buffer **Qiagen Catalog #19076** and mix thoroughly by vortexing. Then add **200 µl** ethanol (96-100%) and mix again thoroughly by vortexing.

> Buffer AL and ethanol can be **premixed** when working with multiple samples, *e.g.*, 1 ml ethanol and 1 ml buffer AL for 10 samples. Is **essential** that the sample, buffer and ethanol are mixed immediately to yield an homogeneous solution. If done this way, you can vortex the samples for **00:05:00** - **00:10:00**

16 Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

17 Carefully transfer the entire lysate to the QIAamp MinElute column (includes a 2 ml collection tube) **without wetting the rim**, close the lid and centrifuge at **6000 x g, Room temperature , 00:01:00** . Place the QIAamp MinElute 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 QIAamp MinElute column is empty.

18 Buffer
Carefully open the QIAamp MinElute column and add **500 µl** AW1 **Qiagen Catalog #19081** **without wetting the rim**. Close the lid and centrifuge at
6000 x g, Room temperature , 00:02:00 , (time considers 30 extra seconds to reach the appropiate speed)
. Place the QIAamp MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through

19 Buffer

Carefully open the QIAamp MinElute column and add **500 µl** AW2 **Qiagen Catalog #19072** **without wetting the rim**. Close the lid and centrifuge at **6000 x g, Room temperature , 00:02:30 , (time considers 30 extra seconds to reach the appropiate speed)**. Place the QIAamp MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.

Contact between the QIAamp MinElute column and the flow-through should be avoided. Seome centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, coming into contact with the QIAamp MinElute column. Take care whem removing the QIAamp MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp MinElute column.

20 Centrifuge at **20000 x g, Room temperature , 00:03:30 , (full speed) (time considers the period it takes the centrifuge to reach maximum speed)**

This step is completely necessary, as ethanol carryover may interfere with some downstream applications.

21 Place the QIAamp MinElute 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 QIAamp MinElute column and apply **35 µl** of Buffer ATE to **the center of the membrane**.

IMPORTANT: Ensure the Buffer ATE is equilibrated at **Room temperature** . If using small elution volumes (< **50 µl**), dispense the Buffer ATE onto the **center of the membrane to ensure complete elution of bound DNA.** Final volume of the eluate will be **5 µl** less than the volume of elution applied to the column.

22 5m

Close the lid and incubate at **Room temperature** for **00:05:00** . Centrifuge **20000 x g, Room temperature , 00:01:30 , (full speed)** .

Incubation time can be as little as 1 minute, but 5 minutes has been observed to increase yield.

23 **go to step #21** and aliquot samples for DNA quality tests and usage in 0.5 ml centrifuge tubes (If used in the next 24 hours, store at **4 °C** , otherwise freeze at **-30 °C** - **-15 °C** .