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

Print Protocol | Print Changes to Previous Version | Print Safety Precautions | Print

High Pure FFPET DNA Isolation Kit

Version: 04
Content version: January 2017

Kit for manual DNA isolation from FFPE tissue for subsequent analysis in PCR, arrays and NG-sequencing.

Cat. No. 06 650 767 001	1 kit 50 isolations

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	white	DNA Tissue Lysis Buffer	▪ For the lysis of cells	10 ml
2	pink	Proteinase K, PCR grade	▪ Lyophilizate ▪ For sample homogenization and inactivation of endogenous nucleases	100 mg
3	green	DNA Binding Buffer	▪ Contains 5 M guanidine HCl ▪ Store protected from light	25 ml
4	black	Wash Buffer I	▪ Contains 5 M guanidine HCl (final concentration after addition of ethanol)	25 ml, add 15 ml absolute ethanol
5	blue	Wash Buffer II		20 ml, add 80 ml absolute ethanol
6	colorless	DNA Elution Buffer		5 × 1,000 µl
7	colorless	Reagent Preparation Buffer		11.5 ml
8		High Pure Filter Tubes	▪ For use of up to 800 µl sample volume	50 polypropylene tubes with two layers of glass fiber fleece
9		Collection Tubes		Three bags with 50 polypropylene tubes (2 ml)

⚠ ***All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.***

The buffers can show a slight yellow color. This will have no impact on the function of the buffer

1.2. Storage and Stability

Storage Conditions (Product)

Kit components are stable at +15 to +25°C until the expiration date printed on the label.

⚠ ***Improper storage at +2 to +8°C (refrigerator) or −15 to −25°C (freezer) will adversely impact nucleic acid purification when precipitates form in the solutions and may result in reduced binding efficiency.***

Storage Conditions (Working Solution)

Solution	Storage
Wash Buffer I	+15 to +25°C
Wash Buffer II	+15 to +25°C
Proteinase K	−15 to −25°C

1.3. Additional Equipment and Reagents Required

- Pipette tips, PCR grade
- Pipettes
- Vortex
- Thermomixer
- Polypropylene tubes, 0.5ml, 1.5 ml, sterile
- Standard tabletop microcentrifuge capable of 16,000 × *g* centrifugal force
- Xylene or alternatively hexadecan
- Ethanol absolute
- Isopropanol

1.4. Application

The High Pure FFPET DNA Isolation Kit uses a fast and optimized technology for the isolation and purification of genomic DNA from formalin-fixed, paraffin-embedded tissue research samples. The quality of DNA from tissue samples is suitable for the following downstream applications:

- Quantitative PCR (qPCR)
- Next generation Sequencing (NGS)

1.5. Preparation Time

Total time	Approx. 3 hours including deparaffinization procedure

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Maximum 10 µm thick sections from formalin-fixed, paraffin-embedded tissue, such as colon, breast, kidney, or lung from mammalian species including human research samples.

⚠ ***Samples containing precipitates must be centrifuged before purification.***

Control Reactions

⚠ ***It is the user's own responsibility to apply an appropriate control concept.***

General Considerations

Handling requirements

⚠ ***Guanidine hydrochloride in Binding Buffer and in Wash Buffer I is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.***
⚠ ***Avoid contact of these buffers with the skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping dry.***
⚠ ***Never store or use the DNA Binding Buffer and Wash Buffer I near human or animal food. Store the DNA Binding Buffer protected from light.***
⚠ ***Do not pool reagents from different lots or from different bottles of the same lot.***
⚠ ***Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions. After first opening store all bottles in an upright position.***
⚠ ***Do not allow the Binding Buffer and/or the Wash Buffer I to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.***

During fixation in formalin, DNA is crosslinked to proteins inter- and introl-molecularly. Formalin-fixed, paraffin-embedded tissue can be stored and handled at +15 to +25 °C.

⚠ ***It is recommended to use sterile disposable polypropylene tubes and tips.***
⚠ ***Always wear gloves during the assay.***

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR setup and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

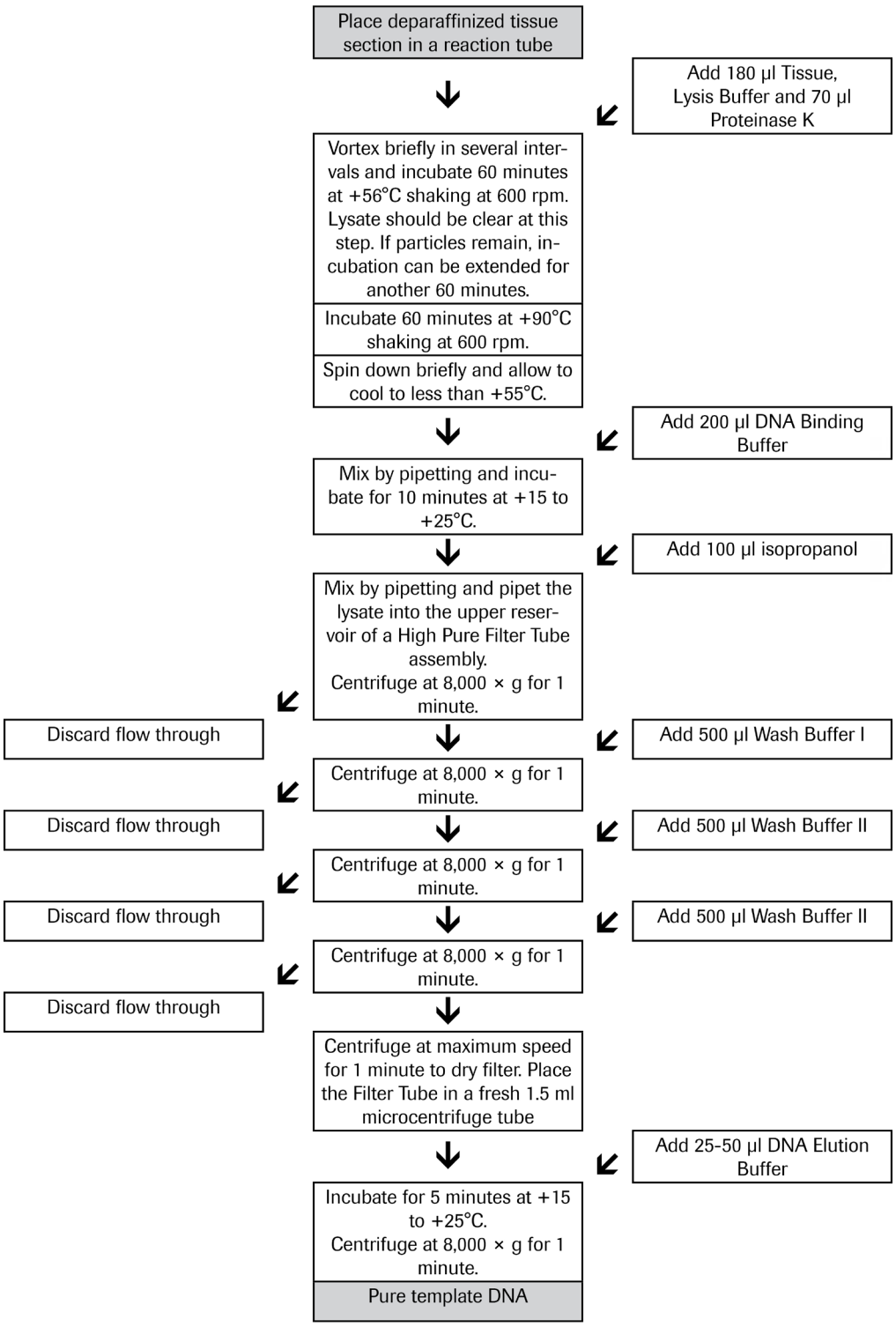
Working Solution

Besides the ready-to-use solutions supplied with this kit, you will need to prepare the following working solutions:

Content	Reconstitution / Preparation	Storage / Stability	For use in...
Proteinase K (Vial 2, pink cap)	Dissolve contents of Vial 2 in 4.5 ml Reagent Preparation Buffer (Vial 7).	▪ Prepare aliquots according to the expected number of samples. Label and store aliquots at −15 to −25°C. ▪ Stable for 12 months	Protocol Step 1: Sample lysis
Wash Buffer I (Vial 4, black cap)	Add 15 ml absolute ethanol. *Label and date vial accordingly after adding ethanol.*	▪ Store prepared Wash Buffer I at +15 to +25°C. ▪ Stable until the expiration date printed on kit label	Protocol Step 7: Washing and elution
Wash Buffer II (Vial 5, blue cap)	Add 80 ml absolute ethanol. *Label and date vial accordingly after adding ethanol.*	▪ Store prepared Wash Buffer II at +15 to +25°C. ▪ Stable until the expiration date printed on kit label	Protocol Steps 8 and 9: Washing and elution

2.2. Protocols

Experimental overview



Deparaffinization The following steps describe the deparaffinization procedure for a single maximum 10 µm thick section of formalin-fixed, paraffin-embedded tissue in a 1.5 ml reaction tube.

1. To one maximum 10 µm section in a 1.5 ml reaction tube, add 800 µl xylene, incubate 5 minutes, and vortex briefly in several intervals.

2. Add 400 µl absolute ethanol and vortex briefly. Centrifuge for 2 minutes at maximum speed (16,000 *x g*) and discard supernatant. Be careful not to dislodge the pellet.

3. Add 1 ml absolute ethanol and vortex briefly. Centrifuge for 2 minutes at maximum speed (16,000 *x g*) and discard supernatant. Be careful not to dislodge the pellet.

4. Blot the tube briefly onto a paper towel to get rid of ethanol residues. Dry the tissue pellet for 10 minutes at +56°C (open tubes). If required, dry for up to a maximum of 20 minutes. Proceed to Step 1 of the DNA isolation protocol.

Alternative Deparaffinization Procedure Using Hexadecan

The following steps describe the deparaffinization procedure for a single maximum 10 µm section of formalin-fixed, paraffin-embedded tissue in a 1.5 ml reaction tube.

1. To one maximum10 µm section in a 1.5 ml reaction tube, add 160 µl hexadecan and vortex for 10 seconds. Incubate for 5 minutes at +56°C.

2. Let cool to +15 to +25°C and add 180 µl of DNA Tissue Lysis Buffer (Vial 1, white cap) and mix (vortex briefly). Centrifuge for 3 minutes at 10,000 *x g*.

3. Add 70 µl Proteinase K working solution (thawed aliquot) carefully to the lower clear aqueous phase and mix by gently pipetting up and down without disturbing the upper organic phase.

4. Incubate 60 minutes at +56°C.

   The lysate should be clear. If particles are still visible, extend incubation time by an additional hour. Spin briefly.

5. Incubate 60 minutes at +90°C. Let cool to +15 to +25°C. Spin down briefly. Transfer all of the lower clear aqueous phase into a new 1.5 ml reaction tube. Proceed to Step 3 of the DNA isolation protocol.

DNA Isolation Protocol

The following protocol describes the DNA isolation from a single maximum 10 µm thick section of formalin-fixed, paraffin-embedded tissue.

1. –To one tissue pellet (deparaffinized as described above), add 180 µl Tissue Lysis Buffer (Vial 1, white cap) and 70 µl Proteinase K working solution (thawed aliquot).
   – Vortex 30 seconds.
   – Spin down briefly.
   – Incubate 60 minutes at +56°C, with shaking at 600 rpm.
   – Spin briefly.

   The lysate should be clear. If particles are still visible, extend incubation time by an additional hour.

❷ Incubate 60 minutes at +90°C, with shaking at 600 rpm.
– Let cool to +15 to +25°C.
– Spin down briefly.

---

❸ Add to tissue lysate: 200 µl DNA Binding Buffer (Vial 3, green cap)
– Mix by pipetting up and down 3 times to get a homogeneous solution.
– Incubate for 10 minutes at +15 to +25°C.

---

❹ Add: 100 µl isopropanol
– Mix by pipetting up and down 3 times.

---

❺ Place High Pure Filter Tube onto High Pure Collection Tube.
– Pipet lysate into the upper reservoir of the High Pure Filter Tube (volume is approximately 550 µl).
– Centrifuge 60 seconds at 8,000 × *g*.

---

❻ Place High Pure Filter Tube onto a new High Pure Collection Tube.
– Discard former High Pure Collection Tube containing flow through.

---

❼ Add 500 µl Wash Buffer I working solution (Vial 4, black cap).
– Centrifuge 60 seconds at 8,000 × *g*. Discard former High Pure Collection Tube containing flow through.
– Place High Pure Filter Tube onto a new High Pure Collection Tube.

---

❽ Add 500 µl Wash Buffer II working solution (Vial 5, blue cap).
– Centrifuge 60 seconds at 8,000 × *g*. Discard flow through and blot the tube briefly onto a fresh paper towel to remove residual Wash Buffer.

---

❾ Add 500 µl Wash buffer II working solution (Vial 5, blue cap).
– Centrifuge 60 seconds at 8,000 × *g*. Discard flow through and blot the tube briefly onto a fresh paper towel to remove residual Wash Buffer.
– Centrifuge for 1 minute at 16,000 × *g* to dry filter fleece completely.
– Place the High Pure Filter Tube into a fresh 1.5 ml reaction tube.

---

❿ Add 25 - 50 µl Elution Buffer (Vial 6, colorless cap) to the center of the fleece without touching the fleece.
– Incubate 5 minutes at +15 to +25°C.
– Centrifuge for 1 minute at 8,000 × *g*.

To get a higher concentrated DNA eluate, the elution volume can be reduced down to 25 µl. DNA yield might me less than when using the 50 µl elution volume.

⓫ The microcentrifuge tube now contains the eluted DNA. Use eluted DNA directly or store at +2 to + 8°C (short term) or –15 to –25°C (long term).

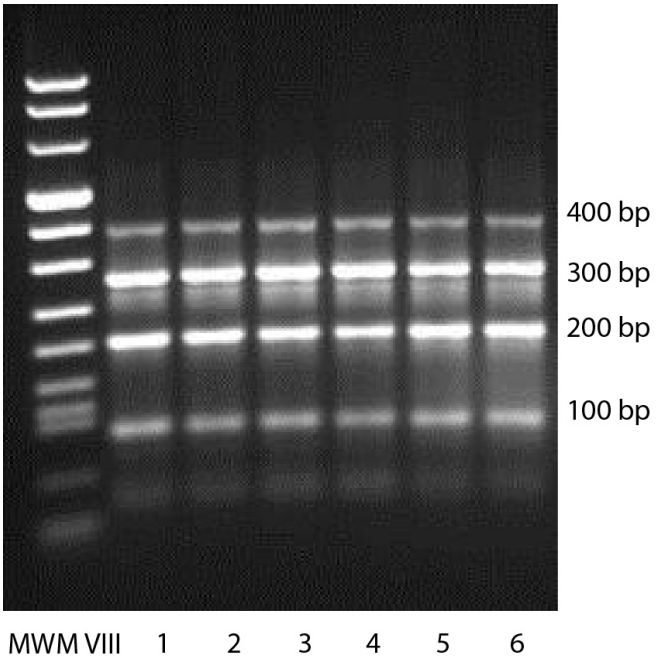
⚠ ***Before photometric determination of the DNA concentration, centrifuge the eluate for 2 minutes at maximum speed, and transfer supernatant to a fresh 1.5 ml reaction tube without disturbing glass fibers at the bottom of the original tube.***

---

3. Results

Purity

Purified DNA is free of nucleases and all cellular and sample components that interfere with PCR, according to the current Quality Control Procedures. As a general rule, it is recommend to design the amplicon length less than approximately 150 bp.
A multiplex PCR is performed which produces PCR products of 100, 200, 300, and 400 bp amplicon length (Van Beers, E. H., et al. (2006)). If all four fragments are obtained, the isolated DNA is qualified and can be used in high end applications, such as aCGH and NG sequencing.



The four bands 100, 200, 300, and 400 bp can be clearly detected, indicating high quality isolated DNA from FFPET using the High Pure FFPET DNA Isolation Kit. The gel contains 2% agarose in 1x TBE buffer.
The purity is determined by calculation of $OD_{260/280\ nm}$.
$OD_{260/280}$ Eluate: 1.7 - 2.0
DNA yield strongly depends on sample type, quality, amount, fixation conditions, and time of storage.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low DNA yield or purity	Kit stored under non-optimal conditions	Store kit at +15 to +25°C at all times after arrival.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness	Store all buffers at +15 to +25°C.
		Close all reagent vials tighly after each use to preserve pH, stability, and freedom from contamination.
		After reconstitution of all lyophilizates, aliquot and store at −15 to −25°C.
	Ethanol not added to Wash Buffer	Add absolute ethanol to buffers before using.
		After addition of ethanol, mix buffers well and store at +15 to +25°C.
		Always mark buffer vials containing added ethanol.
	Reagents and samples not completely mixed	Always mix the sample tube well after addition of each reagent.
		For alternative deparaffinization, perform Step 3 and mix by gently pipetting up and down.
	Isopropanol not added to the lysate in Step 4	Addition of 100 µl isopropanol to the lysate is necessary to promote efficient binding of RNA to the glass fibers.
Tissue homogenate is viscous and difficult to pipet, low DNA yield	Insufficient tissue homogenization	Continue with incubation at +56°C in Step 1 of the DNA isolation protocol until full homogenization of FFPE tissue section is observed.
Clogged Filter Tube	Too much starting material	Do not use more than two 5 µm or one 10 µm tissue section.
		Continue with incubation at +56°C in Step 1 of the DNA isolation protocol until full homogenization of FFPE tissue section is observed.
OD_{260nm} value of eluate too high	Glass fibers, which might coelute with nucleic acids, scatter light	① Remove High Pure Filter Tube from tube containing the eluted sample and centrifuge sample tube for 2 minutes at maximum speed. ② Transfer supernatant to a fresh 1.5 ml reaction tube with-out disturbing glass fibers at the bottom of the original tube.
Samples “pop” out of wells in agarose gels	Eluate contains ethanol (from the Wash Buffer)	① After the wash step, do not let the flow through touch the bottom of the High Pure Filter Tube. ② Empty collection tube, reinsert filter tube in emptied collection tube, and recentrifuge for 30 seconds.

5. Additional Information on this Product

5.1. Test Principle

FFPE tissue samples are disrupted and homogenized during incubation with Tissue Lysis Buffer and Proteinase K. Nucleic Acids (NA) bind in the presence of a chaotropic salt specifically to the surface of glass fibers pre-packed in the High Pure Purification Filter Tube.
DNA is purified in a series of rapid “wash-and-spin” steps to remove salts, proteins, and cellular components. Finally, a low-salt elution releases the DNA from the glass fiber. The process does not require DNA precipitation or organic solvent extraction, ideal for rapidly purifying many samples simultaneously.

① Samples are disrupted in Tissue Lysis Buffer and homogenized during an incubation with Proteinase K.

② In the presence of chaotropic salt, nucleic acids (NA) bind specifically to the surface of glass fibers pre-packed in the High Pure Filter Tube.

③ Bound nucleic acids are washed, and thereby purified of salts, proteins, and other impurities.

④ For the final step, a low-salt elution releases the DNA from the glass fibers.

5.2. References

- Van Beers, E. H. et al. - A multiplex PCR predictor for aCGH success of FFPE samples British J. of Cancer (2006) *Br J Cancer.* **94** (2), 333-337

5.3. Quality Control

Sections from a pre-characterized FFPET block are deparaffinized and DNA isolated according to the Instructions for Use. The optical density is measured at 260 nm and 280 nm, and the DNA concentration and puritiy is determined by calculation. A function test of the isolated DNA in real-time qPCR is performed using the human myostatin gene as target. All quality control critieria have to be fullfilled by meeting predetermined targets.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Trademarks

HIGH PURE is a trademark of Roche.
All third party product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

6.5. Regulatory Disclaimer

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

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

Visit **lifescience.roche.com**, to download or request copies of the following **Materials**:
- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

To call, write, fax, or email us, visit **lifescience.roche.com** and select your home country to display country-specific contact information.

0117.06671306001 ⓒ

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany