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

High Pure FFPET RNA Isolation Kit

Version: 06
Content Version: October 2020

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

Cat. No. 06 650 775 001 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	red	RNA Tissue Lysis Buffer	▪ For the lysis of cells	6 ml
2	pink	Proteinase K, PCR grade	▪ Lyophilizate ▪ For sample homogenization and inactivation of endogenous nucleases	2 × 100 mg
3	green	RNA Binding Buffer	▪ Contains 5 M guanidine thiocyanate *Store protected from light*	20 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	white	DNase I	▪ Lyophilizate ▪ Contains 4 kU DNAse I ▪ For digestion of residual DNA	100 mg
7	white	DNase Incubation Buffer (1x)		6 ml
8	colorless	RNA Elution Buffer	▪ Water, PCR Grade	5 × 1,000 µl
9	colorless	Reagent Preparation Buffer		11.5 ml
10		High Pure Filter Tubes		50 polypropylene tubes with two layers of glass fiber fleece
11		Collection Tubes		3 bags containing 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
DNase I (reconstituted)	-15 to -25°C

1.3. Additional Equipment and Reagent required

- Pipette tips, PCR grade
- Pipettes
- Vortex
- Thermomixer
- Polypropylene tubes, 1.5 ml, sterile
- Standard tabletop microcentrifuge capable of 16,000 × g centrifugal force
- Xylene
- Ethanol absolute
- SDS, 10% (ready-to-use)
- Water, PCR Grade

1.4. Application

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

- RT-PCR
- Gene Expression Array
- Next Generation Sequencing
- PreAmplification

1.5. Preparation Time

Assay Time

Total time	Approx. 2.5 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, lung, and kidney from mammalian origin including human research samples.
- Macrodissected samples (typically 3 – 5 µm) or biopsy material, such as colon, breast, lung, and kidney from mammalian origin including human research samples.
- Laser Microdissection (LMD) samples (typically 3 – 5 µm), for example lung and breast FFPE tissue from mammalian origin including human research samples (minimum 1 mm^2, 25 µl elution volume is recommended for microdissected sample material).

General Considerations

⚠ ***Guanidine thiocyanate in RNA Binding Buffer and Guanidine hydrochloride 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 RNA Binding Buffer and Wash Buffer I near human or animal food. Store the 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 RNA 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, intracellular RNases become inactivated. However, RNA is degraded and crosslinked to proteins inter- and introl-molecularly. For this reason, formalin-fixed, paraffin-embedded tissue can be stored and handled at +15 to +25 °C.

⚠ ***It is recommended to use sterile dispoable 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.

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

Working Solution

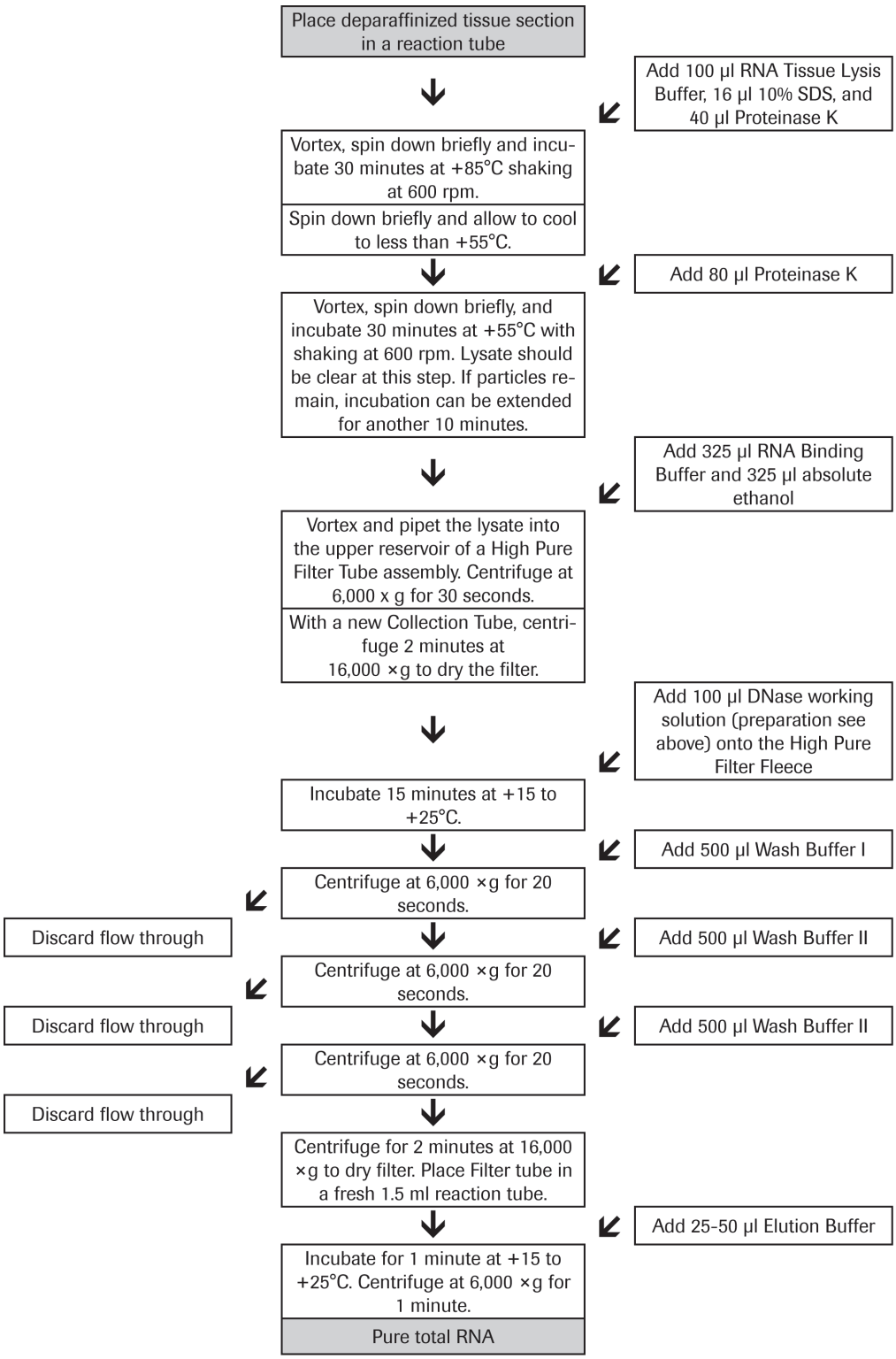
Besides the ready-to-use solutions supplied with this kit, you will need t prepare the following working solutions:

Content	Reconstition / Preparation	Storage / Stability	For use in ...
Wash Buffer I (Vial 4, black cap)	▪ Add 15 ml absolute ethanol.	▪ Store prepared Wash Buffer I at +15 to +25°C. ▪ Stable until the expiration date printed on kit label	Step 7 RNA Isolation Protocol, washing and elution
Wash Buffer II (Vial 5, blue cap)	▪ Add 80 ml absolute ethanol.	▪ Store prepared Wash Buffer II at +15 to +25°C. ▪ Stable until the expiration date printed on kit label	Steps 8 and 9 RNA Isolation protocol, washing and elution
Proteinase K (Vial 2, pink cap)	▪ Dissolve contents of bottle 2 in 4.5 ml Reagent Preparation Buffer (Vial 9). ▪ Prepare aliquots according to the expected number of samples.	▪ Label and store aliquots at −15 to −25°C. ▪ Stable for 12 months	Steps 1 and 2 RNA Isolation Protocol, sample lysis
DNase I (Vial 6, white cap)	▪ Dissolve contents of bottle 6 in 740 µl Reagent Preparation Buffer (Vial 9). ▪ Prepare aliquots according to the expected number of samples.	▪ Label and store aliquots at −15 to −25°C. ▪ Stable for 12 months	Preparation of DNase working solution
DNase working solution	▪ Prepare required amount for expected number of samples.	⚠ ***Always prepare the DNase working solution fresh for each extraction round! Do not store!***	Step 6 RNA Isolation Protocol, washing and elution

Required amount for N (number of) RNA isolations:
(N+1) × 90 µl DNase Incubation Buffer (Vial 7, white) + (N+1) × 10 µl DNase I (dissolved, see above).

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 xylene, and vortex briefly in several intervals.

2. Add 400 µl absolute ethanol and vortex briefly. Centrifuge for 2 minutes at maximum speed (16,000 × *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 × *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 + 55°C (open tubes). If required, dry for up to a maximum of 20 minutes. Proceed to Step 1 of the RNA isolation protocol.

Alternative Procedure for Macrodissection

The following steps describe the deparaffinization procedure for a single section of formalin-fixed, paraffin-embedded tissue on a microscope slide (typically 3 - 5 µm).

1. Place the slide in a xylene bath and incubate for 2 minutes.

2. Change bath and incubate the slide for an additional 2 minutes in xylene.

3. Tap off excess liquid and place the slide into ethanol abs. for 1 minute.

4. Change bath and incubate the slide for 1 minute in ethanol 96%.

5. Change bath and incubate the slide for 1 minute in ethanol 70%.

6. Change bath and incubate the slide for 1 minute in H_2O (Water PCR Grade).

7. Add 5 - 10 µl RNA Tissue Lysis Buffer (Vial 1, red cap) on top of the section.
   ⚠ ***Volume needs to be subtracted from amount of RNA Tissue Lysis Buffer in Step 1, RNA Isolation Protocol.***

8. Remove the deparaffinized section from the slide by using a sterile single-use scalpel and place it into a 1.5 ml reaction tube.

⚠ ***To avoid scattering of the tissue, remove the section from the microscope slide before it has dried.***

Proceed to Step 1 of the RNA isolation protocol.

Alternative Procedure for Laser Microdissections (LMD)

The following steps describe the deparaffinization procedure for a single section of formalin-fixed, paraffin-embedded tissue on a microscope slide suit-able for microdissection (typically 3 - 5 µm).

⚠ ***Depending on the type of laser capture microscope, special slides may have to be used (e.g., membrane slides).***

1. Place the slide in a xylene bath and incubate for 2 minutes.

2. Change bath and incubate the slide for an additional 2 minutes in xylene.

3. Tap off excess liquid and place the slide into ethanol abs. for 1 minute.

4. Change bath and incubate the slide for 1 minute in ethanol 96%.

5. Change bath and incubate the slide for 1 minute in ethanol 70%.

6. Change bath and incubate the slide for 1 minute in H_2O (Water PCR Grade).

7. Dry the tissue on the slide for approximately 1 hour at +15 to +25°C.

8. Microdissect your sample material according to the manufacturers' instructions. It is recommended to collect your microdissected material in liquid. You can use RNA Tissue Lysis Buffer (Vial 1, red cap).

   ⚠ ***Volume needs to be subtracted from amount of RNA Tissue Lysis Buffer in Step 1, RNA Isolation Protocol.***

9. After capturing the sample material, proceed with Step 1 of the RNA isolation protocol

ℹ *Optionally: Samples can be frozen at -15 to -25 °C until further processing.*

⚠ ***For RT-qPCR analysis, approximately 1 - 2 mm² of laser microdissected material is required if you are not using an additional pre-amplification step.***

RNA Isolation Protocol

The following protocol describes the RNA isolation from a single maximum 10 µm thick section of formalin-fixed, paraffin-embedded tissue or a macro-/ microdissected tissue sample (typically 3 - 5 µm).

Depending on the size of the embedded tissue, the number of preparations could be pooled in Step 4:

- For 10 µm sections: 2
- For macrodissected samples: 3
- For LMD: >3 preparations

1. To one tissue pellet (deparaffinized as described above), add 100 µl RNA Tissue Lysis Buffer (Vial 1, red cap), 16 µl 10% SDS, and 40 µl Proteinase K working solution (thawed aliquot).
   Reagents can be added separatly or as a mix.
   - Vortex several seconds.
   - Spin down briefly.
   - Incubate 30 minutes at +85°C, with shaking at 600 rpm.
   - Spin briefly.
   - Cool to less than +55°C before adding Proteinase K.

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2. Add 80 µl Proteinase K (from thawed aliquot; discard unused enzyme).
   - Vortex several seconds.
   - Spin down briefly.
   - Incubate 30 minutes at +55°C, while shaking at 600 rpm.
   - Spin down briefly.

   The lysate should be clear at this step. If particles remain, incubation can be extended for another 10 minutes.

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3. Add to tissue lysate: 325 µl RNA Binding Buffer (Vial 3, green cap), 325 µl absolute ethanol
   - Vortex several seconds.
   - Spin down briefly.

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4. - 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 900 µl).
   - Centrifuge 30 seconds at 6,000 × *g*.

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5. - Place High Pure Filter Tube onto a new High Pure Collection Tube.
   - Centrifuge 2 minutes at 16,000 × *g* to dry filter fleece completely.

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6. - Place High Pure Filter Tube onto new High Pure Collection Tube.
   - Add 100 µl DNase working solution (preparation see above) onto the High Pure Filter Tube fleece without touching the fleece.
   - Incubate 15 minutes at +15 to +25°C.

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7. - Add 500 µl Wash Buffer I working solution (Vial 4, black cap).
   - Centrifuge 20 seconds at 6,000 × *g*. Discard flow through.

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8. - Add 500 µl Wash buffer II working solution (Vial 5, blue cap).
   - Centrifuge 20 seconds at 6,000 × *g*. Discard flow through

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9. - Add 500 µl Wash buffer II working solution (Vial 5, blue cap).
   - Centrifuge 20 seconds at 6,000 × *g*. Discard flow through.
   - Centrifuge for 2 minutes at 16,000 × *g* to dry filter fleece completely.
   - Place the High Pure Filter Tube into a fresh 1.5 ml reaction tube.

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⑩ - Add 25 - 50 µl RNA Elution Buffer (Vial 8, colorless cap) to the center of the fleece tube without touching the fleece.
- Incubate 1 minute at +15 to +25°C.
- Centrifuge for 1 minute at 6,000 × *g*.

A standard elution volume of 50 µl can be applied. If you are working with small amounts of sample material and low RNA yields are expected, it is recommended to reduce the elution volume to 25 µl.

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⑪ The microcentrifuge tube now contains the eluted RNA. Use eluted RNA directly or store at +2 to +8°C (short term) or -70°C (long term).

⚠ ***Before photometric determination or capillary gel electrophoresis of the RNA, 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 RNA is free of DNA, nucleases, and all cellular and sample components that interfere with RT-PCR, according to the current Quality Control Procedures.
The purity is determined by calculation of $OD_{260/280nm}$.
Ratio $_{260/280}$ Eluate: 1.8 - 2.0

RNA Integrity and size distribution

The size of the isolated RNA fragments is analyzed using the Agilent Bioana-lyzer. Ninety percent of RNA fragments are greater than 100 nucleotides (exemplary data). The RIN number is usually very low for FFPET-derived RNA samples due to fragmentation. RIN should be >1.4 as a general rule, but even RNA samples with no RIN may work if your PCR amplicons are small.

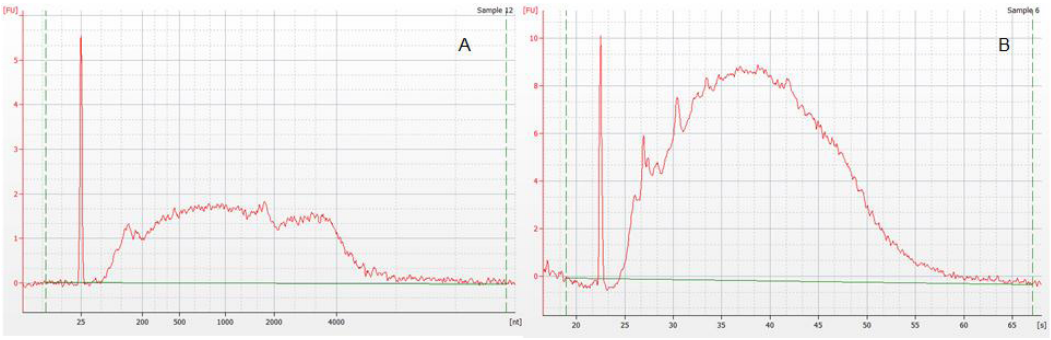

Fig. 1: Electropherograms obtained from FFPET- derived RNA generated by the Agilent 2100 Bioanalyzer.
A: The trace shows the result of a purification from a Xenograft (KPL4, BrCa) 5 µm section in an elution volume of 25 µl. Corresponding RIN is 2.4, OD_{260nm} measurement shows a concentration of 77 ng/µl.

B: The trace shows the results of a purification from 2 NSCLC (Non-small-cell lung carcinoma) 10 µm sections (pooled in step 4 of the RNA Isolation Protocol) in an elution volume of 50 µl. Corresponding RIN is 2.2, OD_{260nm} measurement shows a concentration of 275 ng/µl. RNA yield and integrity strongly depends on sample type, quality, amount, fixation conditions, and time of storage.

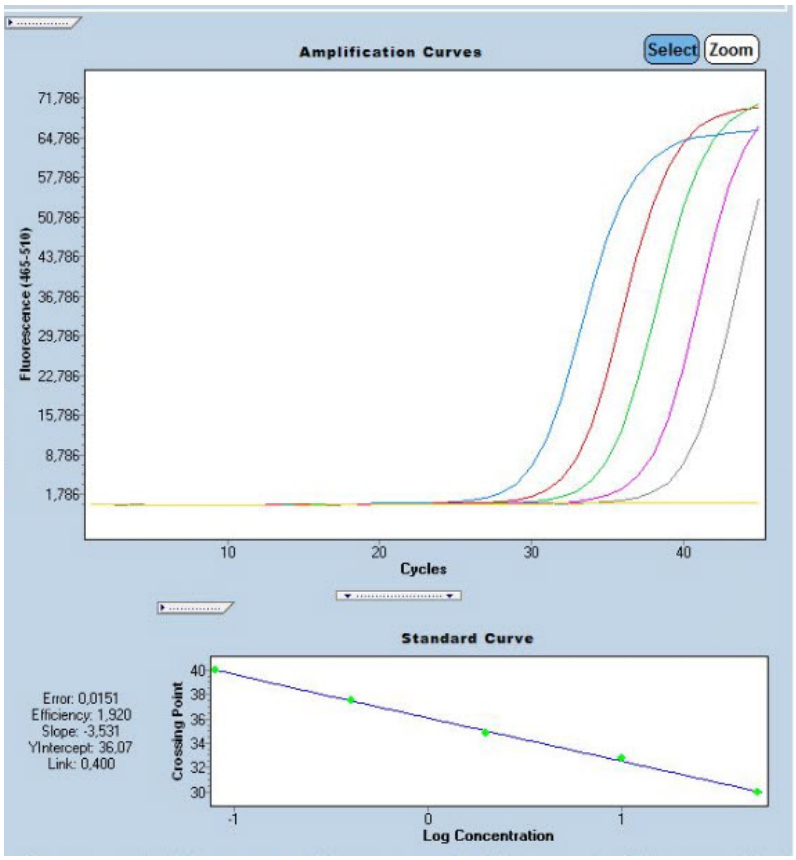

Fig. 2: Typical results for a RT-qPCR experiment.
cDNA synthesis has been performed using the Transcriptor Universal cDNA Master. The expression level of the reference gene ALAS 1 (assayID 102108) was determined in a serial dilution of RNA (50 ng to 0.08 ng/PCR) isolated from NSCLC (Non-small-cell lung carcinoma) samples.

4. Troubleshooting

Observation	Possible cause	Recommendation
Low RNA 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 tightly after each use to preserve pH, stability, and freedom from contamination.
		After reconstitution of all lyophilizates, aliquot and store at -15 to -25°C.
	Precipitates in the Tissue Lysis Buffer, SDS, or Proteinase K	Do not work on ice as SDS precipitates below room temperature.
	Precipitates in the SDS stock solution	SDS precipitates below room temperature, therefore dissolve precipitates in a water bath at +37°C.
	Ethanol not added to Wash Buffer	Add absolute ethanol to buffers before using.
		After adding 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.
	Ethanol not added to the lysate in Step 3	Addition of 0.5 volume of absolute ethanol to the lysate is necessary to promote selective binding of RNA to the glass fibers.
	High level of RNase activity	Be careful to create an RNase-free working environment.
		Process starting material immediately or store at -80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store immediately at -80°C.
	RNA concentration too low for your application	Reduce the elution volume to a minimum of 25 µl.
OD $_{260nm}$ value of eluate too high	Glass fibers, which might coelute with nucleic acids, scatter light	▪ Remove High Pure Filter Tube from tube containing the eluted sample and centrifuge sample tube for 1 minutes at maximum speed. ▪ Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Samples “pop” out of wells in agarose gels	Eluate contains ethanol (from the Wash Buffer)	▪ After the wash step, do not let the flow through touch the bottom of the High Pure Filter Tube. ▪ 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 RNA 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.
RNA is purified in a series of rapid “wash-and-spin” steps to remove salts, proteins, and cellular components.
Residual DNA is digested on the column by adding DNase I.
Finally, a low-salt elution releases the RNA from the glass fiber. The process does not require RNA precipitation or organic solvent extraction, ideal for rapidly purifying many samples simultaneously.

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

① 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.

② To eliminate residual DNA, an on-column DNase I digestion is performed

③ Bound RNA is washed, and thereby purified of salts, proteins, and other impurities.

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

5.2. Quality Control

Sections from a pre-characterized FFPET block are deparaffinized and isolated as described. RNA yield is determined by measuring the optical density at 260 nm and RNA purity is determined by the OD ratio 260/280 nm. The integrity of the purified sample material is confirmed using the Agilent RNA Pico Kit. Absence of contaminating genomic DNA is examined by a PCR on a LightCycler® System without a reverse transcription step according to the current Quality Control Procedures.

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

New information added related to the REACH Annex XIV

6.3. Trademarks

LIGHTCYCLER is a trademark of Roche.
All other 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
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- Certificates of Analysis
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