Instructions for Use
Life Science Kits & Assays

innuPREP FFPE total RNA Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-2050010 10 reactions
845-KS-2050050 50 reactions
845-KS-2050250 250 reactions

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Contents

1 Introduction

1.1 Intended use

The kit has been designed as a very efficient tool for fast isolation of total RNA (including microRNA) from FFPE samples. The kit can be used with different amount of FFPE tissue samples / sections, up to a maximum amount of tissue: 50 mg. For detail information see Product specifications (→ “Product specifications”, p. 10).

The extraction procedure is based on a new patented chemistry and combines lysis of FFPE tissue samples with subsequent binding of nucleic acids onto the surface of a Spin Filter membrane. After several washing steps the nucleic acids are eluted from the membrane by using RNase-free water. Extraction chemistry and extraction protocol are optimized to get maximum of yield.

The innuPREP FFPE total RNA Kit is not for use with other starting materials as describe above and the kit performance has not been evaluated for other starting materials.

Consult instruction for use

This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

REF
Catalogue number.

N

Content
Contains sufficient reagents for <N> tests.

Storage conditions
Store at room temperature.

Consult instructions for use
This information must be observed to avoid improper use of the kit and the kit components.

Use by

Lot number
The number of the kit charge.

Manufactured by

For single use only

Note / Attention
Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with arrow (e.g. → "Notes on the use of this manual" p. 4).
- Working steps are numbered.

2 Safety precautions

Note

Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.

Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin immediately with a large amount of water.

For single use only!

This kit is made for single use only!

Attention!

Don't eat or drink components of the kit!

The kit shall only be handled by educated personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. Analytik Jena AG has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely, but cannot be excluded completely. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Clinical sample must always be considered as potentially infectious. Samples from risk patients must always be labeled and handled under consequent safety conditions. Please observe the federal, state and local safety and environmental regulations.

Observe the usual precautions for applications using extracted nucleic acids. All materials and reagents used for RNA isolation should be free of RNases.

Attention!

Do not add bleach or acidic components to the waste after sample preparation!

Note

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

For more information, please ask for the material safety data sheets (MSDS's).

3 Storage conditions

The innuPREP FFPE total RNA Kit should be stored dry, at room temperature (14 - 25 °C) and is stable for at least 12 months under these conditions. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming. For further information see table kit components (→ “Kit components” p. 7).

4 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. The components of each innuPREP FFPE total RNA Kit were tested by isolation of total RNA from a FFPE sample of human origin and subsequent amplification of a human specific target sequence.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the innuPREP FFPE total RNA Kit or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

5 Product use and warranty

The user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. Analytik Jena AG kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by the Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

Note

For research use only!

6 Kit components

Important

Store lyophilized Proteinase K at 4 °C!

Divide dissolved Proteinase K into aliquots and storage at – 20 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically!

Storage conditions

All other components are stored at room temperature.

	Σ 10	Σ 50	Σ 250
REF	845-KS-2050010	845-KS-2050050	845-KS-2050250
Lysis Solution MA	5 ml	25 ml	120 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution	for 7 x 1.5 ml working solution
Washing Solution C	8 ml (ready-to-use)	30 ml (ready-to-use)	135 ml (ready-to-use)
Washing Solution BS	2 ml (final vol. 20 ml)	10 ml (final vol. 100 ml)	18 ml (final vol. 180 ml)
RNase-free water	2 ml	3 x 2 ml	25 ml
Spin Filter (red)	10	50	5 x 50
Receiver Tubes (2.0 ml)	50	5 x 50	25 x 50
Elution Tubes (1.5 ml)	10	50	5 x 50
Manual	1	1	1
Initial steps	• Add 18 ml of 96-99.8% ethanol to the bottle Washing Solution BS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 0.3 ml of ddH_2O, mix thoroughly and store as described above!	• Add 90 ml of 96-99.8 % ethanol to the bottle Washing Solution BS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described above!	• Add 162 ml of 96-99.8 % ethanol to the bottle Washing Solution BS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described above!

7 Recommended steps before starting

- Pre-heat thermal mixer or water bath to 65 °C, followed by 80 °C.
- Ensure that the Washing Solution BS and Proteinase K have been prepared according to the instruction (→ "Kit components" p. 7).
- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

8 Components not included in the kit

- 1.5 ml reaction tubes (safe lock)
- 2.0 ml reaction tubes; optional
- 96 – 99.8 % ethanol absolute, biological grade, no denatured ethanol
- ddH_2O
- DNase I Digestion (→ "Related Products" p. 19).

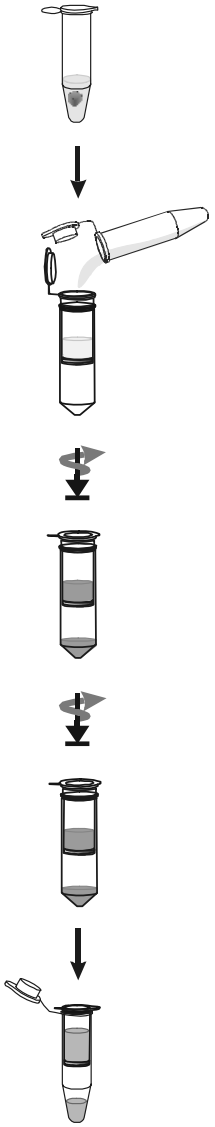
9 Extraction procedure

9.1 Summary

The kit has been designed as a tool for very fast and efficient isolation of total RNA (including microRNA) from FFPE samples. The extraction procedure is based on a new kind of chemistry, which combines an efficient lysis step with a subsequent effective binding of total RNA on a Spin Filter surface, followed by washing of the bound RNA and final elution of the RNA. The recovery and the quality of isolated RNA are excellent. The new kind of chemistry allows the isolation of RNA from FFPE samples without the deparaffinization step and thereby exclude the use of toxic and hazardous components like octane or xylene.

The extraction process is finished within less than 1.5 hour. The isolated RNA is suitable for reverse transcription, amplification reaction and other amplification based further downstream applications.

9.2 General extraction principle



Lysis of starting material

Centrifugation of sample and removal of paraffin and residues of tissue (not shown in the overview)

Binding of RNA on Spin Filter (red)

Washing of the bound RNA

Elution of RNA

10 Product specifications

1. Starting material:

- FFPE (formalin fixed paraffin embedded) tissue samples
- Approx. 12 mg (approx. 18 µl) paraffin correspond to:

≈ 6 sections of 10 µm thickness and each of 300 mm^2 area

≈ 4 sections of 10 µm thickness and each of 400 mm^2 area

≈ 3 sections of 10 µm thickness and each of 600 mm^2 area

≈ 2 sections of 10 µm thickness and each of 900 mm^2 area

≈ 1 sections of 10 µm thickness and each of 1.800 mm^2 area

- Maximum amount of tissue: 50 mg

Note

Depending on the amount of starting material, it is possible to proceed more sections than indicated above. In such case, it is the costumer's responsibility to validate the innuPREP FFPE total RNA Kit for this new purpose.

2. Time for isolation:

Approximately 1.5 hour (all steps included)

3. Typical yield:

- Depends on type, quantity and amount of starting material

The extracted total RNA (including microRNA) can be used for a wide range of different molecular biology applications.

11 Example of application

1. Procedure:

- Extraction of total RNA from FFPE tissue samples with different amount of starting material
- Spectrophotometric measurement of all samples
- Amplification of a microRNA sequence (hsa-miR-16-5p)
- Electrophoresis in Agilent 2100 Bioanalyzer (Small RNA Kit)
- Electrophoresis in formaldehyde agarose gel

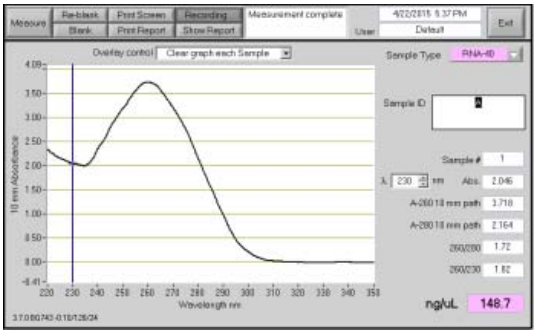
Sample ID	No. of sections	Section thickness	Weight of tissue	Paraffin surface
A	4	10 µm	~ 2 mg	1.200 mm^2
B	2	10 µm	~ 2 mg	600 mm^2
C	1	10 µm	~ 2 mg	300 mm^2
D	3	10 µm	~ 6 mg	900 mm^2

2. Results:

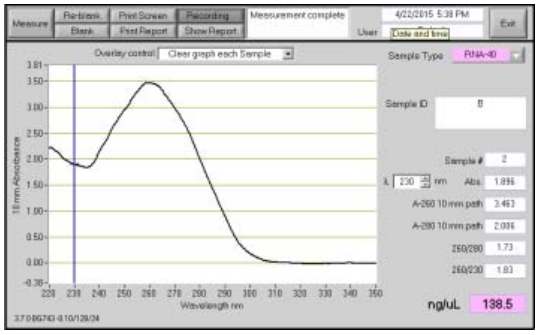
After the extraction process, the isolated RNA (eluted in 50 µl RNase-free water) was measured by spectrophotometric method, subsequent amplification for a microRNA sequence by Real-Time-PCR and controlled by Bioanalyzer using Small RNA Kit:

	Spectrophotometric measurement				Agilent Bioanalyzer	
Sample ID	**Ø Concentration (total RNA)**	**Ratio $A_{260/280}$**	**Ratio $A_{260/230}$**	**Mean Ct value**	**Ø Concentration (small RNA)**	**miRNA**
A	148.7 ng/µl	1.72	1.82	24.35	10.98 ng/µl	15 %
B	138.5 ng/µl	1.73	1.83	24.31	10.76 ng/µl	16 %
C	136.1 ng/µl	1.73	1.80	24.49	11.18 ng/µl	16 %
D	490.5 ng/µl	1.74	1.90	23.12	14.95 ng/µl	28 %

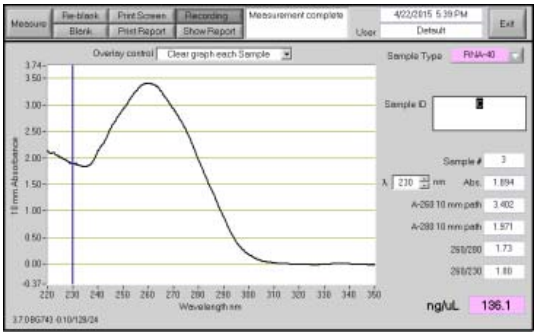
Spectrophotometric analysis of sample A - D:



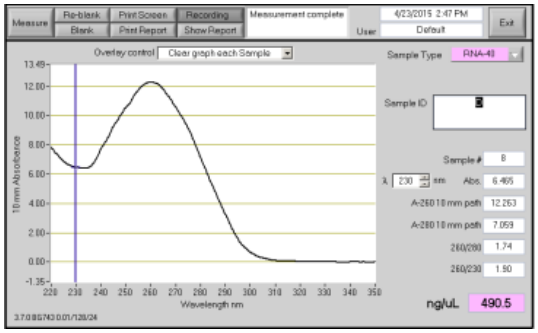
Sample A: 4 sections, 2 mg tissue, 1.200 mm^2 paraffin.



Sample B: 2 sections, 2 mg tissue, 600 mm^2 paraffin.

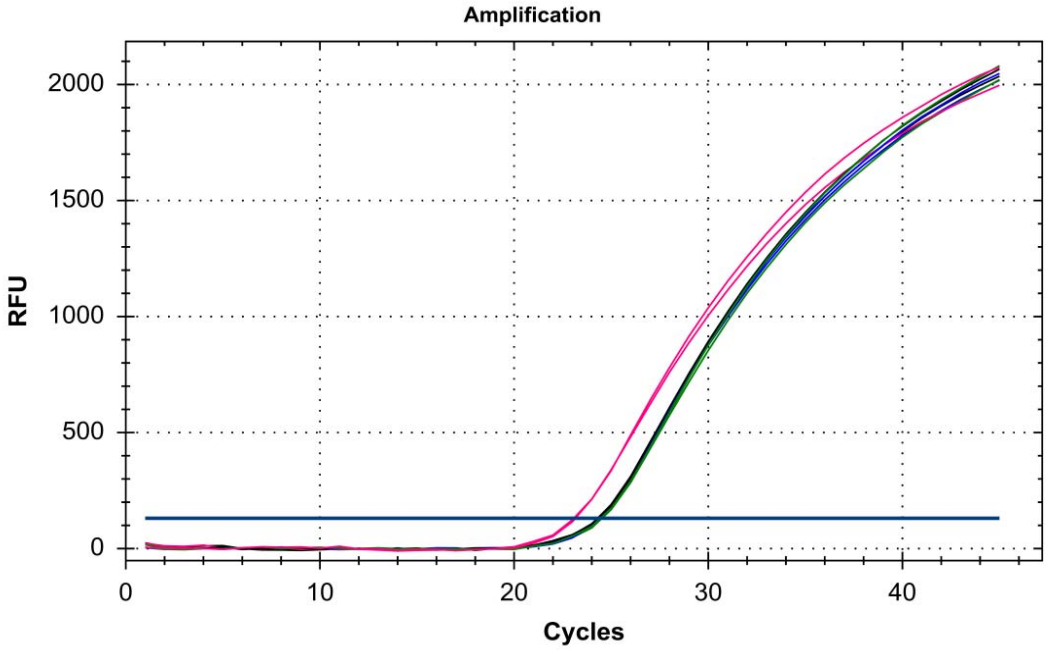


Sample C: 1 section, 2 mg tissue, 300 mm^2 paraffin.



Sample D: 3 sections, 6 mg tissue, 900 mm^2 paraffin.

Analysis of sample A up to sample D by Real-Time-PCR (hsa-miR-16-5p):

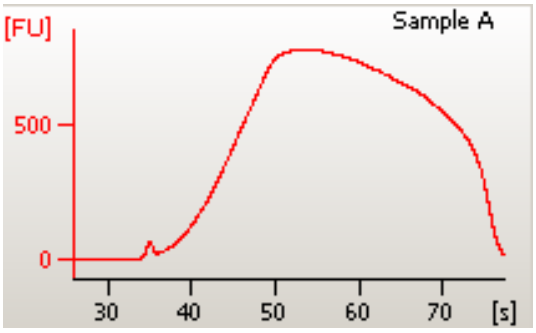


The Ct values of different FFPE tissue samples correspond to the yield as shown above. Sample A (■), sample B (■), sample C (■) and sample D (■).

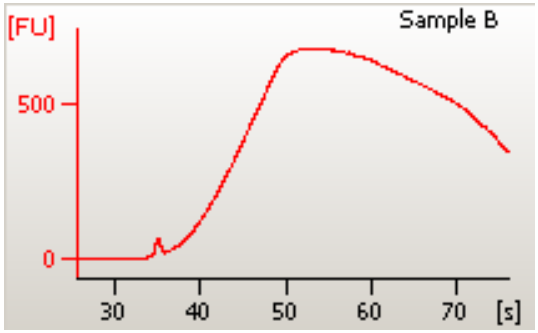
Analysis of sample A up to sample D using Small RNA Kit (Agilent Bioanalyzer):

	Sample Name	Status	Result Label
1	Sample A	✓	15 % miRNA; Concentration: 10976.60 pg/µl
2	Sample B	✓	16 % miRNA; Concentration: 10755 pg/µl
3	Sample C	✓	16 % miRNA; Concentration: 11177.90 pg/µl
4	Sample D	✓	28 % miRNA; Concentration: 14945.90 pg/µl

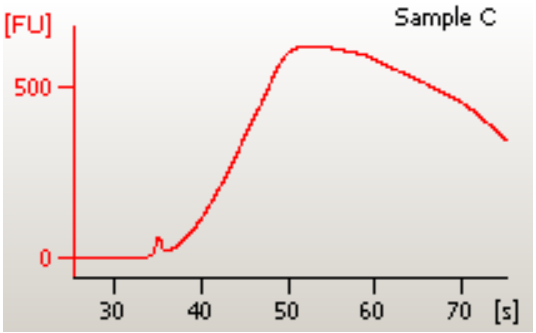
Sample A - D: Overview on quantity of miRNA.



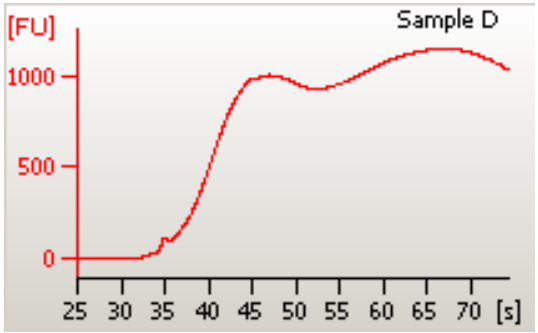
Sample A: 4 sections, 2 mg tissue, 1.200 mm^2 paraffin.



Sample B: 2 sections, 2 mg tissue, 600 mm^2 paraffin.

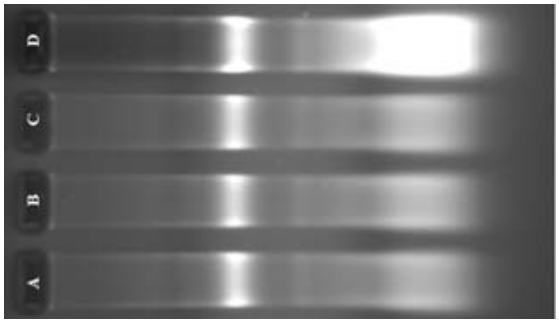


Sample C: 1 section, 2 mg tissue, 300 mm^2 paraffin.



Sample D: 3 sections, 6 mg tissue, 900 mm^2 paraffin.

Electrophoresis in 1.2 % formaldehyde agarose gel of sample A up to sample D:



Sample A - D: Electrophoresis of all samples.

12 Protocol: RNA isolation from paraffin embedded tissue samples

1. Place the **FFPE material** into a 1.5 ml or 2.0 ml reaction tube and centrifuge the reaction tube at maximum speed for 1 minute.

   Note: For correct sample amount see Product specifications (→ “Product specifications”, p. 10).

2. Open the reaction tube and add **400 µl Lysis Solution MA and 40 µl Proteinase K** to the sample, mix vigorously by pulsed vortexing for 10 sec.

   Important note: The FFPE material has to be completely covered by Lysis Solution MA. Fragments that not covered by Lysis Solution MA will be not completely lysed and may influence quality and purity of extracted RNA. If necessary, push them down by the end of tips or spin down briefly to remove drops from the lid!

3. Incubate the reaction tube at 65 °C for 30 minutes in a thermal mixer under continuous shaking at 1.000 rpm.

   Note: We recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively: vortex the sample 3 - 4 times during the incubation. No shaking will reduce the lysis efficiency and leads to lower RNA yield.

   Important note: If the residual tissue sample is still visible after 30 minutes, it is possible to prolong the incubation step until the tissue is completely lysed. Longer incubation time can increase RNA yield, but it is not obligatory, since also samples with not completely lysed tissue carry very high yield of RNA.

4. After lysis step centrifuge the sample at maximum speed for 3 minutes. Open the tube and transfer the supernatant into a new 1.5 ml reaction tube (RNase-free and Safe Lock tube). Avoid disturbing the pellet and transfer of solid particles of paraffin.

   Note: Samples with high amount of paraffin will form a strong, solid paraffin layer on the surface. Pierce it by the end of tips and transfer all supernatant from underneath of paraffin layer. Samples with lower amounts of paraffin will not form visible layer. In this case transfer all liquid samples (without pellet) together with upper cloud if formed.

   Note: Samples with small amount of tissue will not form a visible pellet.

   Note: If the pellet is very soft, transfer all supernatant and as little as possible of the pellet (without solid paraffin particles) into a new 1.5 ml reaction tube and repeat the centrifugation at maximum speed for 3 minutes. Transfer the supernatant into a new 1.5 ml reaction tube without disturbing the pellet.

   Note: For samples containing very large amount of tissue (brown color after incubation at 65 °C), step 4 should be repeated.

5. Incubate the reaction tube at 80 °C for 30 minutes in a thermal mixer under continuous shaking at 1.000 rpm.

   Important note: Do not shorten or prolong the incubation time at 80 °C. Shorter incubation time may result in lower efficiency of decrosslinking and longer may degrade RNA.

6. Centrifuge the sample at maximum speed for 3 minutes and transfer the supernatant into a new 1.5 ml reaction tube. Avoid disturbing the pellet and transfer of paraffin layer if formed.

   Note: Pellet and solid paraffin layer will be not formed if the sample was proceed well after first incubation step at 65 °C (can appear a “cloud” in the upper part of sample).

7. Add **600 µl of ethanol absolute (96-99 %)** to the sample, mix vigorously by pulsed vortexing for 10 seconds or pipetting up and down several times.

   Note: It is important that the sample and the ethanol absolute are mixed vigorously to get a homogeneous solution.

8. Apply **600 µl of the sample** onto a Spin Filter (red) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

9. Transfer the residual sample onto the Spin Filter (red) located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

   Note: After this step DNase treatment is optional. The removal of DNA from the sample by DNase I could lead in partial loss of RNA.

10. Open the Spin Filter and add **500 µl Washing Solution C**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

    Discard the filtrate and place the Spin Filter back into the 2.0 ml Receiver Tube.

11. Open the Spin Filter and add **650 µl Washing Solution BS**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

    Discard the filtrate and place the Spin Filter into a new 2.0 ml Receiver Tube.

12. Open the Spin Filter and add **650 µl ethanol absolute (96-99 %)**, close the cap and centrifuge at 10.000 x g (~12.000 rpm) for 1 minute.

    Discard the filtrate and place the Spin Filter into a new 2.0 ml Receiver Tube.

13. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

    Note: To ensure to remove all traces of ethanol, the lid of the spin filter may remain opened during centrifugation.

10. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50 µl RNase-free water**. Incubate at room temperature for 1 minute. Centrifuge at 10.000 x g (~12.000 rpm) for 1 minute. A second elution step will increase the yield of extracted DNA.

Note

The RNA can be eluted with a lower or a higher volume of RNase-free water (depends on the expected yield of total RNA). Elution with lower volumes of RNase-free water increases the final concentration of RNA.

A second elution step using the eluat from the first elution step can significantly increase the yeld of RNA of samples with small amount of tissue.

Store the extracted RNA at +4 °C. For long time storage placing at –20 °C is recommended.

13 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
• Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.
Low amount of extracted RNA	
• Insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
• Incomplete elution	Prolong the incubation time with RNase-free water or repeat elution step once again. Take a higher volume of RNase-free water.
• Insufficient mixing with ethanol absolute	Mix sample with ethanol absolute by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
• Too long incubation at 80 °C	Do not prolong (or shorten) sample incubation in 80 °C.
Low concentration of extracted RNA	
• Too much RNase-free water	Elute the RNA with lower volume of RNase-free water.
Degraded or sheared RNA	
• Incorrect storage of starting material	Ensure that the starting material has been stored under proper conditions!
• Old material insufficient	FFPE material often contains degraded RNA.
DNA contaminations of extracted RNA	DNase I digestion
Bad Ratio at $A_{260/230}$ and $A_{260/280}$	
• Carryover of paraffin or pellet	Take carefully out all solution by piercing the paraffin layer using a 100 µl pipette after centrifugation step (step 4 & 6). Avoid carryover of paraffin and pellet to the next tube! If carry over occurred, repeat centrifugation and transfer step.

Problem / probable cause	Comments and suggestions
Sample diffuses out from the electrophoresis gel	
• Ethanol was not completely removed from the Spin Filter	Centrifuge at maximum speed for 3 minuntes (step 12) with open lid of the Spin Filter. Prolong the centrifugation step at maximum speed before elution step (step 12). Incubate eluted samples in elution tube with opened lied at 37 °C for 30 minuntes.

14 Related Products

Name	Amount	Order No.
Nucleid acid purification		
innuPREP Proteinase K	6 mg	845-CH-0010006
	30 mg	845-CH-0010030
innuPREP DNase I Digest Kit	10 rxn	845-KS-5200010
	50 rxn	845-KS-5200050
	250 rxn	845-KS-5200250
innuPREP DNase I	5.000 Kunitz Units	845-KS-5210005
	10.000 Kunitz Units	845-KS-5210010
Products for Reverse Transcription		
innuSCRIPT One Step RT-PCR Probe Kit	100 rxn	845-RT-7000100
	200 rxn	845-RT-7000200
innuSCRIPT One Step RT-PCR SyGreen Kit	100 rxn	845-RT-6000100
	200 rxn	845-RT-6000200
innuSCRIPT Reverse Transcriptase [25 U/µl]	50 rxn (1.250 U)	845-RT-5000050
	200 rxn (5.000 U)	845-RT-5000200
Products for PCR & Gel Electrophoresis		
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
innuTaq RED DNA Polymerase (1 U/µl)	500 U	845-EZ-2000500
innuTaq Hot-A DNA Polymerase (5 U/µl)	500 U	845-EZ-3000500
innuTaq UltraPure DNA Polymerase (5 U/µl)	500 U	845-EZ-6000500
50x inNucleotide Mix (12,5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
25 mM $MgCl_2$ - Solution	3x 1.5 ml	845-AS-1000015
50 mM $MgCl_2$ - Solution	3x 1.5 ml	845-AS-1010015
PCR-grade H_2O	2.0 ml	845-AS-1800002
	5x 2.0 ml	845-AS-1800010
innuMIX rapidPCR MasterMix	100 rxn	845-AS-1600100
	200 rxn	845-AS-1600200
innuMIX Standard PCR MasterMix	100 rxn	845-AS-1700100
	200 rxn	845-AS-1700200

Name	Amount	Order No.
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
	200 rxn	845-AS-1400200
innuSTAR 100 bp DNA Ladder Express	500 µl	845-ST-1010100
	5x 500 µl	845-ST-1010500
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500
6x Loading Dye Bromophenol Blue	3x 1.0 ml	845-ST-3010003
	6x 1.0 ml	845-ST-3010006
6x Loading Dye Orange G	3x 1.0 ml	845-ST-4010003
	6x 1.0 ml	845-ST-4010006
Products for qPCR		
innuMIX qPCR MasterMix Probe	100 rxn	845-AS-1200100
	200 rxn	845-AS-1200200
innuMIX qPCR MasterMix SyGreen	100 rxn	845-AS-1300100
	200 rxn	845-AS-1300200

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Pictures: Analytik Jena AG
Subjects to changes in design and scope of delivery as well as further technical development!

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