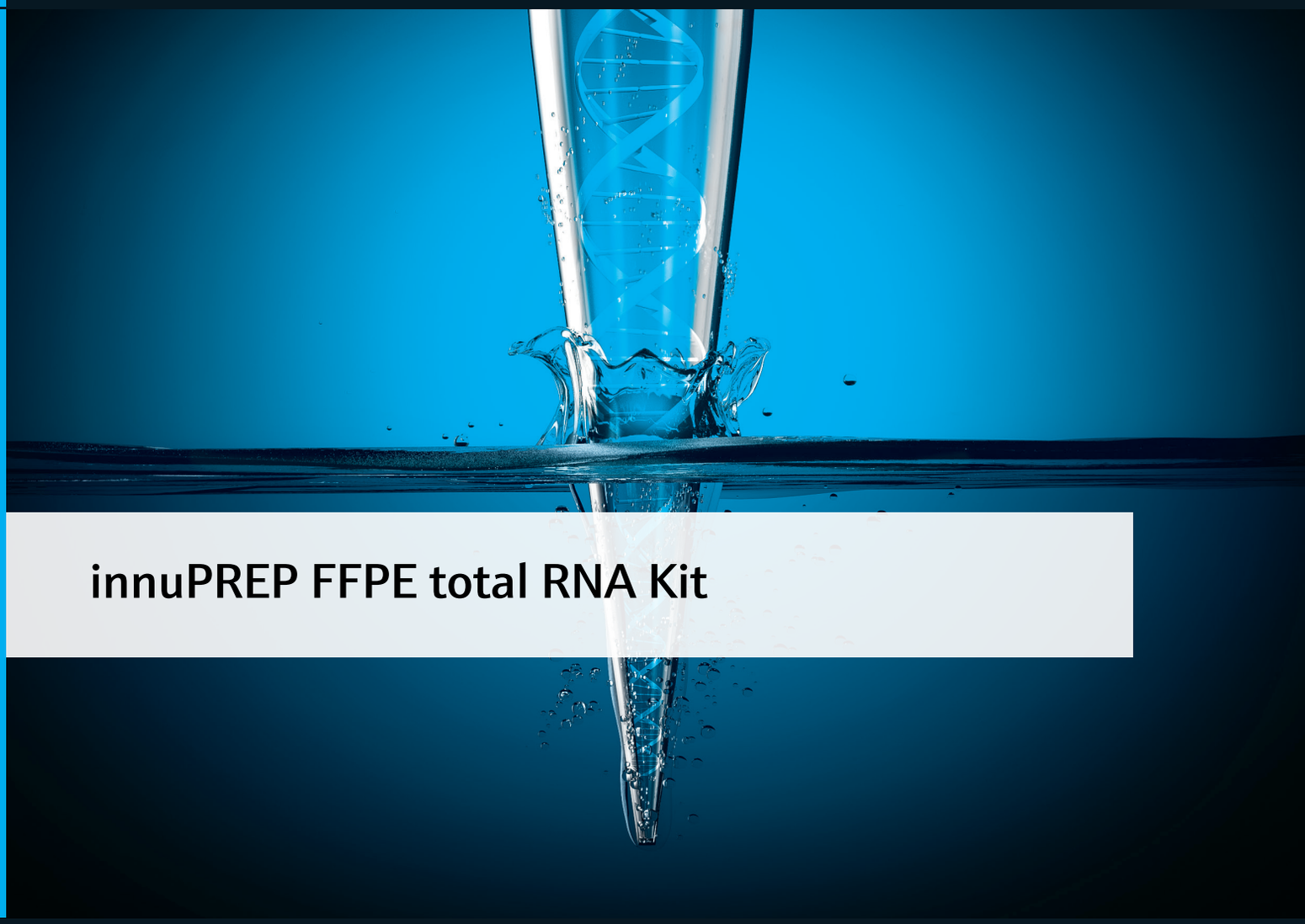
Instructions for Use
Life Science Kits & Assays



innuPREP FFPE total RNA Kit

Innuscreen GmbH

Order No.:

845-KS-2050010 10 reactions
845-KS-2050050 50 reactions

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This documentation describes the state at the time of publishing.
It needs not necessarily agree with future versions. Subject to change!

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1 Introduction

1.1 Intended use

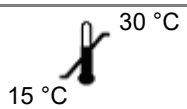
The innuPREP FFPE total RNA Kit has been designed for fast isolation of total RNA (including microRNA) from FFPE samples. The kit can be used with different amount of FFPE tissue samples / sections, up to a maximum amount of tissue: 50 mg.

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.

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully before 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 and the kit

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

Symbol	Information
	**REF** Catalogue number.
	**Content** Contains sufficient reagents for <N> reactions.
	**Storage conditions** Store at room temperature or shown conditions respectively.
	**Consult instructions for use** This information must be observed to avoid improper use of the kit and the kit components.
	**Expiry date**
	**Lot number** The number of the kit charge.
	**Manufactured by** Contact information of manufacturer.
	**For single use only** Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the kit 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 an arrow (e.g. → „Notes on the use of this manual and the kit“ p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE

Read through this chapter carefully before 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 with a large amount of water immediately.

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 personnel 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. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or 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 on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com.

3 Storage conditions

The kit is shipped at ambient temperature.

Upon arrival store lyophilized and dissolved Proteinase K at 4 °C to 8 °C.

The innuPREP FFPE total RNA Kit should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

If there are any precipitates within the provided solutions solve these precipitates by careful warming. Before every use make sure that all components have room temperature.

4 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This product has been produced and tested in an ISO 13485 certified facility.

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 IST Innuscreen GmbH products, please do not hesitate to contact us.

For technical support or further information in Germany please contact info.innu@ist-ag.com. For other countries please contact your local distributor.

5 Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use“ p. 2), (→ “Product specifications“ p. 8). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH 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 IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE

The kit is for research use only!

6 Kit components

6.1 Included kit components

	Σ 10	Σ 50
REF	845-KS-2050010	845-KS-2050050
Lysis Solution MA	5 ml	25 ml
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution
Washing Solution C	8 ml	30 ml
Washing Solution BS (conc.)	2 ml	10 ml
RNase-free Water	2 ml	3 x 2 ml
Spin Filter	10	50
Receiver Tubes	50	5 x 50
Elution Tubes	10	50
Manual	1	1

6.2 Components not included in the kit

- 1.5 ml reaction tubes
- 2.0 ml reaction tubes; optional
- Ethanol 96–99.8 %; non denatured or methylated
- ddH_2O
- DNase I Digestion

7 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 section 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 90 minutes (all steps included)

3. Typical yield:
   - Depends on the type, the quantity and the amount of the starting material

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

8 Initial steps before starting

- Add the indicated amount of absolute ethanol to **Washing Solution BS (conc.)**, mix thoroughly and store as described above.

845-KS-2050010	Add 18 ml ethanol to 2 ml Washing Solution BS (conc.).
845-KS-2050050	Add 90 ml ethanol to 10 ml Washing Solution BS (conc.).

- Add the indicated amount of ddH_2O to **Proteinase K**, mix thoroughly and store as described above.

845-KS-2050010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-2050050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

- Pre-heat thermal mixer or water bath to 65 °C, followed by 80 °C.
- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

9 General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free Water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four or more hours before use. Autoclaving alone will not inactivate many RNases completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C and then it has to be autoclaved or heated to 100 °C for 15 minutes to remove residual DEPC.

- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5 % SDS), thoroughly rinsed with RNase-free Water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free ddH_2O.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

10 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. 8)

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.

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

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.

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 at maximum speed for 3 minutes and transfer the supernatant into a new 1.5 ml reaction tube. Avoid disturbing the pellet and transferring any paraffin layer that may have formed

NOTE

Pellet and solid paraffin layer do not form if the sample is well proceeded after first incubation step at 65 °C (a “cloud” can appear in the upper part

of the 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 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 passed through the Spin Filter completely, 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 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 the step DNase treatment is optional. The removal of DNA from the sample by DNase I could lead to 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 back into the 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 back into the 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 open during centrifugation.

14. 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 eluate from the first elution step can significantly increase the yield of RNA of samples with small amount of tissue.

Store the extracted RNA at +4 °C. For long time storage placing at −22 °C to -18 °C is recommended.

11 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 before transferring the sample to the Spin Filter.
Too long incubation at 80 °C	Do not prolong (or shorten) sample incubation at 80 °C.
Low concentration of extracted RNA	
Too much RNase-free Water	Elute the RNA with lower volume of RNase-free Water.
Total RNA degraded or sheared	
RNA source inappropriately handled or stored	Ensure that the starting material has been stored under proper conditions!
Old material insufficient	FFPE material often contains degraded RNA
DNA contamination	
DNA contamination	apply DNase I digestion
Bad Ratio at $A_{260/230}$ and $A_{260/280}$	
Carryover of paraffin or pellet	After the centrifugation step (step 4 and 6), carefully remove the entire solution by piercing through the paraffin layer with a 100 µl pipette. If carryover occurred repeat centrifugation and transfer step.

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