Instructions for Use
Life Science Kits & Assays

innuPREP MP Basic FFPE RNA Kit

Innuscreen GmbH

Order No.:

845-KS-4910010 10 reactions

845-KS-4910050 50 reactions

Publication No.: HB_KS-4910_e_220322

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

Manufacturer and Distributor:
IST Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin · Germany
Made in Germany!
Phone +49 30 9489 3380
Fax +49 30 9489 3381
info.innu@ist-ag.com

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1 Introduction

1.1 Intended use

The innuPREP MP Basic FFPE RNA 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 magnetic particles 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 excludes the use of toxic and hazardous components like octane or xylene. The extraction process is finished within less than 2 hours. The isolated RNA is suitable for reverse transcription, amplification reaction and other amplification based further downstream applications.

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	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.
15 °C 30 °C	**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 kit components.
	Expiry date
LOT	**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” p.3)
- Working steps are numbered.

2 Safety precautions

NOTE
Read through this chapter carefully before use 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. IST Innuscreen GmbH 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.

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** and **MAG Suspension F** at 4 °C to 8 °C.

All other components of the innuPREP MP Basic FFPE 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.

For further information see chapter "Kit components" p. **Fehler! Textmarke nicht definiert.**.

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 MP Basic FFPE RNA Kit or other IST Innuscreen GmbH products, please do not hesitate to contact us.

For technical support or further information in Germany please dial +49 30 9489 3380. 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 (→ "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-4910010	845-KS-4910050
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
MAG Suspension F	250 µl	1.1 ml
Washing Solution C	8 ml	30 ml
Washing Solution BS (conc.)	2 ml	5 ml
RNase-free Water	2 ml	3 x 2 ml
Elution Tubes	10	50
Manual	1	1

6.2 Components not included in the kit

- 1.5 ml reaction tubes (safe lock)
- 2.0 ml reaction tubes; optional
- Magnetic trap
- 96–99.8 % ethanol, non denatured or methylated
- ddH_2O
- DNase I; optional

7 Product specifications

Starting material

- FFPE (formalin fixed paraffin embedded) tissue samples
- Approx. 12 mg (apprx. 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 MP Basic FFPE RNA Kit for this new purpose.

Time for isolation

- Approximately 2 hours (all steps included)

Typical yield

- Depends on the type, quantity and amount of starting material

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

NOTE

Avoid freezing and thawing of starting material

8 Initial steps before starting

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

845-KS-4910010	Add 0.3 ml ddH_2O to lyophilized Proteinase K.
845-KS-4910050	Add 1.5 ml ddH_2O to lyophilized Proteinase K.

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

845-KS-4910010	Add 18 ml ethanol to 2 ml Washing Solution BS (conc.).
845-KS-4910050	Add 45 ml ethanol to 5 ml Washing Solution BS (conc.).

- Pre-heat thermal mixer or water bath to 65 °C, followed by 80 °C and 60 °C.
- Centrifugation steps should be carried out at room temperature.

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 min 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 **450 µl Lysis Solution MA** and **40 µl Proteinase K** to the sample, mix vigorously by pulsed vortexing for 10 sec.

NOTE

The FFPE material has to be covered by Lysis Solution MA completely. Fragments that are not covered by Lysis Solution MA will not be lysed completely and can affect the quality and purity of the 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.

ATTENTION!

If the residual tissue sample is still visible after 30 minutes, it is possible to prolong the incubation step until the tissue is lysed completely. A longer incubation time can increase the RNA yield, but is not absolutely necessary since samples with incompletely lysed tissue also have a very high RNA yield.

4. Incubate the sample at room temperature for 3 minutes and 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 Safe Lock tube). Avoid disturbing the pellet and transferring solid paraffin particles.

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.

NOTE

Do not shorten or prolong the incubation time at 80 °C. A shorter incubation time may result in lower efficiency or decrosslinking, a longer one may degrade the RNA.

6. Incubate the sample for 3 minutes at room temperature and centrifuge the tube at maximum speed for 3 minutes. Transfer the supernatant into a new 1.5 ml rection tube. Avoid disturbing the pellet and transferring the paraffin layer it forms.

NOTE

Pellet and solid parrafin layer will not be formed if the sample was proceeded well after first incubation step at 65 °C (a “cloud” could 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 to mix the sample and the ethanol vigorously to get a homogeneous solution.

8. Add **10 µl of MAG Suspension F** to the sample, close the reaction tube and invert it for 1 minute (approx. 60 times). Place the reaction tube in a **magnetic trap** and invert the trap 10 times to collect all magnetic particles. Open the reaction tube and remove all supernatant (also from the lid) by pipetting. Be careful to not disturb the magnetic particles pellet. Discard the supernatant.

NOTE

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

9. Remove the reaction tube from the magnetic trap and add **500 µl of Washing Solution C**. Close the reaction tube and invert it for 1 minute (approx. 60 times). Place the reaction tube in a **magnetic trap** and invert the trap 10 times to collect all magnetic particles. Open the reaction tube and remove all supernatant (also from the lid) by pipetting. Be careful to not disturb the magnetic particles pellet. Discard the supernatant.

10. Remove the reaction tube from the magnetic trap and add **650 µl Washing Solution BS**. Close the reaction tube and invert it for 1 minute (approx. 60 times). Place the reaction tube in a **magnetic trap** and invert the trap 10 times to collect all magnetic particles. Open the reaction tube and remove all supernatant (also from the lid) by pipetting. Be careful to not disturb the magnetic particles pellet. Discard the supernatant.

11. Remove the reaction tube from the magnetic trap and add **650 µl ethanol absolute (96 99 %).** Close the reaction tube and invert it for 1 minute (approx. 60 times). Place the reaction tube in a magnetic trap and invert the trap 10 times to collect all magnetic particles. Open the reaction tube and remove all supernatant (also from the lid) by pipetting. Be careful to not disturb the magnetic particles pellet. Discard the supernatant.

12. Remove the reaction tube from the magnetic trap and incubate the opened reaction tube at 60 °C for 3 minutes in a thermal mixer to remove residuals of ethanol.

NOTE

If the pellet of magnetic particles contains more ethanol, the incubation time can be prolonged. Be careful to not overdry the pellet.

13. Add **100 µl of RNase-free water** and dissolve the pellet by pipetting and/or vortexing. Incubate the reaction tube at room temperature for 3 minutes. Pipette the sample 10 times up and down. Place the reaction tube in a **magnetic trap** to collect the magnetic particles. Transfer the supernatant containing eluted RNA into an Elution Tube.

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.

Store the extracted RNA at +2 °C to 6 °C. For long time storage placing at −22 °C to −18 °C is recommended.

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Little or no RNA eluted	
Insufficient disruption or homogenization	Increase lysis time! Reduce amount of starting material. Overloading reduces yield!
Incomplete elution	Prolong the incubation time with RNase-free Water to 5 minutes or repeat elution step. Increase the volume of RNase-free Water.
Total RNA does not perform well in downstream applications	
Ethanol carryover during elution	Increase the time for removing ethanol.
Salt carryover during elution	Ensure that Washing Solution BS is completely removed.

IST Innuscreen GmbH
Robert-Rössle-Str.10
13125 Berlin · Germany

Phone +49 30 9489 3380
Fax +49 30 9489 3381

info.innu@ist-ag.com

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