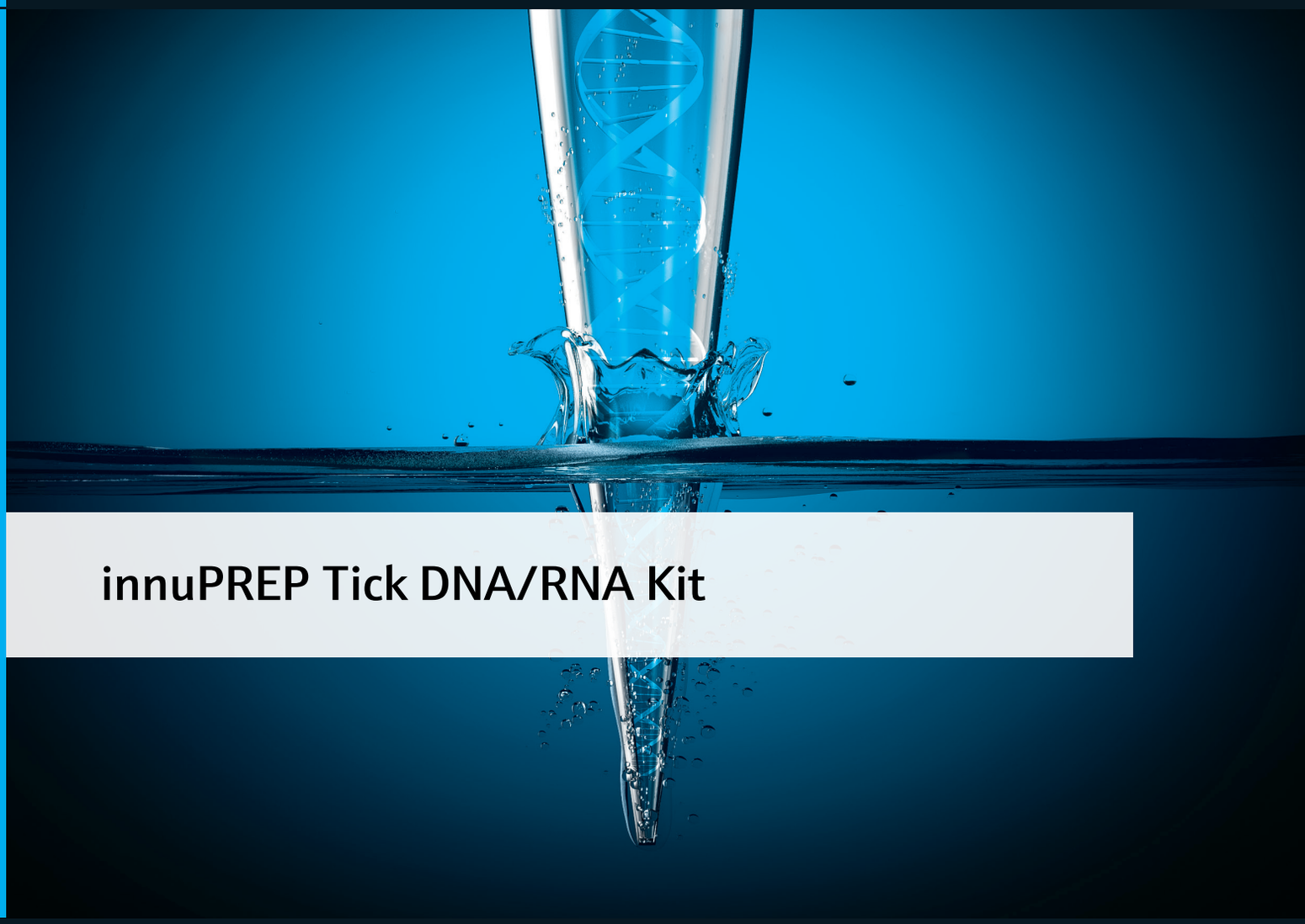
Instructions for Use
Life Science Kits & Assays



innuPREP Tick DNA/RNA Kit

iST Innuscreen GmbH

Order No.:

845-KS-5100010	10 reactions
845-KS-5100050	50 reactions

Publication No.: HB_KS-5100_e_250106

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 Tick DNA / RNA Kit** has been designed for simple, reliable and fast isolation of genomic DNA and RNA from ticks. The isolation procedure starts with the mechanical homogenization of the tick followed by purification of nucleic acids using spin column technology.

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> tests.
15 °C 30 °C	**Storage conditions** Store at room temperature, unless otherwise specified.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the 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 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 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 is designed to be handled only 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 is to be used with potential infectious human 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 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.

All other components of the **innuPREP Tick DNA/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.

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

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 Tick DNA/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 (→ “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 equivalent regulations required in other countries.

All products sold by the 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 Components included in the kit

	Σ 10	Σ 50
REF	845-KS-5100010	845-KS-5100050
Lysis Tube P	10	50
Lysis Solution RL	6 ml	30 ml
Washing Solution HS (conc.)	6 ml	30 ml
Washing Solution LS (conc.)	3 ml	2 x 8 ml
Elution Buffer	2 ml	6 ml
RNase-free Water	2 ml	6 ml
Spin Filter D	10	50
Spin Filter R	10	50
Receiver Tubes (2.0 ml)	2 x 40	8 x 50
Elution Tubes (1.5 ml)	2 x 10	2 x 50
Manual	1	1

6.2 Components not included in the kit

- DNase I (optional)
- 1.5 ml reaction tubes
- Lysozyme (optional)
- ddH_2O
- TE-Buffer (10 mM Tris-HCl; 1 mM EDTA; pH 8,0) (optional)
- Ethanol (70 %, 96–99.8 %); non-denatured or methylated
- SpeedMill or other type of homogenizer

7 Initial steps before starting

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

845-KS-5100010	Add 6 ml ethanol to 6 ml Washing Solution HS (conc.).
845-KS-5100050	Add 30 ml ethanol to 30 ml Washing Solution HS (conc.).

- Add the indicated volume of absolute ethanol to **Washing Solution LS (conc.)** and mix thoroughly. Always keep the bottle firmly closed!

845-KS-5100010	Add 12 ml ethanol to 3 ml Washing Solution LS (conc.).
845-KS-5100050	Add 32 ml ethanol to 8 ml Washing Solution LS (conc.).

- Centrifugation steps should be performed at room temperature.
- Avoid freezing and thawing of starting materials.

8 Product specifications

1. Starting material:
   Whole ticks

2. Time for isolation:
   Approximately 15–40 minutes.

3. Typical yield:
   Not determined. The yield depends on the amount and quality of the 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: DNA and RNA extraction from ticks

10.1 Homogenization of the tick using SpeedMill or another homogenizer

NOTE
To maximize the final yield of DNA and total RNA a complete homogenization of the ticks is important!

1. Transfer the whole tick into a **Lysis Tube P** and add **100 µl Lysis Solution RL**. Close the Lysis Tube P firmly.
2. Place the **Lysis Tube P** into the sample holder of the SpeedMill as described in the user manual of the device.
3. Homogenization: 4 minutes

NOTE
If the tick is not homogenized completely, continue the homogenization process. In case of using another homogenizer based on beads, please follow the recommendations of the manufacturer!

4. Add **300 µl Lysis Solution RL** to the homogenized tick and incubate the sample under continuous shaking for 30 minutes at room temperature.

10.2 Extraction procedure

1. After incubation of the sample, centrifuge the Lysis Tube P at maximum speed to spin down unlysed material. Place a Spin Filter D into a Receiver Tube. Transfer the supernatant of the lysed sample onto the Spin Filter D. Centrifuge at 10,000 x g for 2 minutes. Centrifuge the sample for 20 minutes at 5.000 x g. If the centrifuge does not allow such a high speed, use the maximum speed. Remove the supernatant as much as possible.

Do not discard the filtrate, because the filtrate contains the RNA!

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

2. Place the Spin Filter D into a new Receiver Tube. The DNA is bound onto Spin Filter D. Processing of the Spin Filter D will be continued after binding of total RNA onto Spin Filter R (→ step 5).

3. Place a Spin Filter R into a new Receiver Tube. Add **350 µl** of **70 % ethanol** to the filtrate from step 1. Mix the sample by pipetting up and down several times. Transfer the sample onto the Spin Filter R. Centrifuge at 10,000 x g for 2 minutes.

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

4. Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube. The total RNA is bound on Spin Filter R. Both Spin Filters (Spin Filter D and R) will be processed in parallel now.

5. Open the Spin Filters D and R, add **500 µl Washing Solution HS** to each, close the caps and centrifuge at 10,000 x g for 1 minute. Discard the Receiver Tubes with the filtrate and place both Spin Filters D and R into new Receiver Tubes.

6. Open the Spin Filters D and R, add **650 µl Washing Solution LS** to each, close the caps and centrifuge at 10,000 x g for 1 minute. Discard the Receiver Tubes with the filtrate and place both Spin Filters D and R into new Receiver Tubes.
7. Centrifuge at 10,000 x g for 2 minutes to remove all traces of ethanol. Discard the Receiver Tubes.
8. Place the Spin Filters D (DNA elution) and Spin Filter R (RNA elution) each into an Elution Tube. Carefully open the caps of the Spin Filters D and R, add **100 µl Elution Buffer** to Spin Filter D and **30–80 µl RNase-free Water** to Spin Filter R. Incubate at room temperature for 2 minutes. Centrifuge at 5,000 x g for 1 minute.

NOTE

Depending on the extracted yield or the needed concentration of DNA or total RNA, it is also possible to elute with different volumes of Elution Buffer/RNase-free Water. A lower volume of Elution Buffer/RNase-free Water increases the concentration of DNA/RNA and a higher volume of Elution Buffer/RNase-free Water leads to an increased yield but a lower concentration of nucleic acids. Please note, that the minimum of RNase-free Water should be 20 µl.

Store nucleic acids at appropriate conditions (RNA at −80 °C and DNA at -22 °C to -18 °C)!

11 Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient disruption or homogenization	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no DNA or total RNA eluted	
Insufficient disruption or homogenization	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 once again.
DNA contamination	
Too much starting material	Reduce amount of starting material.
Incorrect lysis of starting material	Use the recommended techniques for lysis of cell pellet. Perform DNase digest of the eluate containing the total RNA or perform an on column DNase digest step after binding of the RNA on Spin Filter R!
Total RNA degraded	
RNA source inappropriately handled or stored	Ensure that the starting material is fresh! Ensure that the protocol, especially the first steps, have been performed quickly.
RNase contamination of solutions; Receiver Tubes, etc.	Use sterile, RNase-free filter tips. Before every preparation clean up the pipette, the devices and the working place. Always wear gloves!
Total RNA does not perform well in downstream applications (e.g. RT-PCR)	
Ethanol carry-over during elution	Increase time for removing of ethanol.
Salt carry-over during elution	Ensure that **Washing Solution HS** and **Washing Solution LS** are at room temperature. Checkup Washing Solution for salt precipitates. If there are any precipitate dissolves these precipitate by carefully warming.

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

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Fax +49 30 9489 3381

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