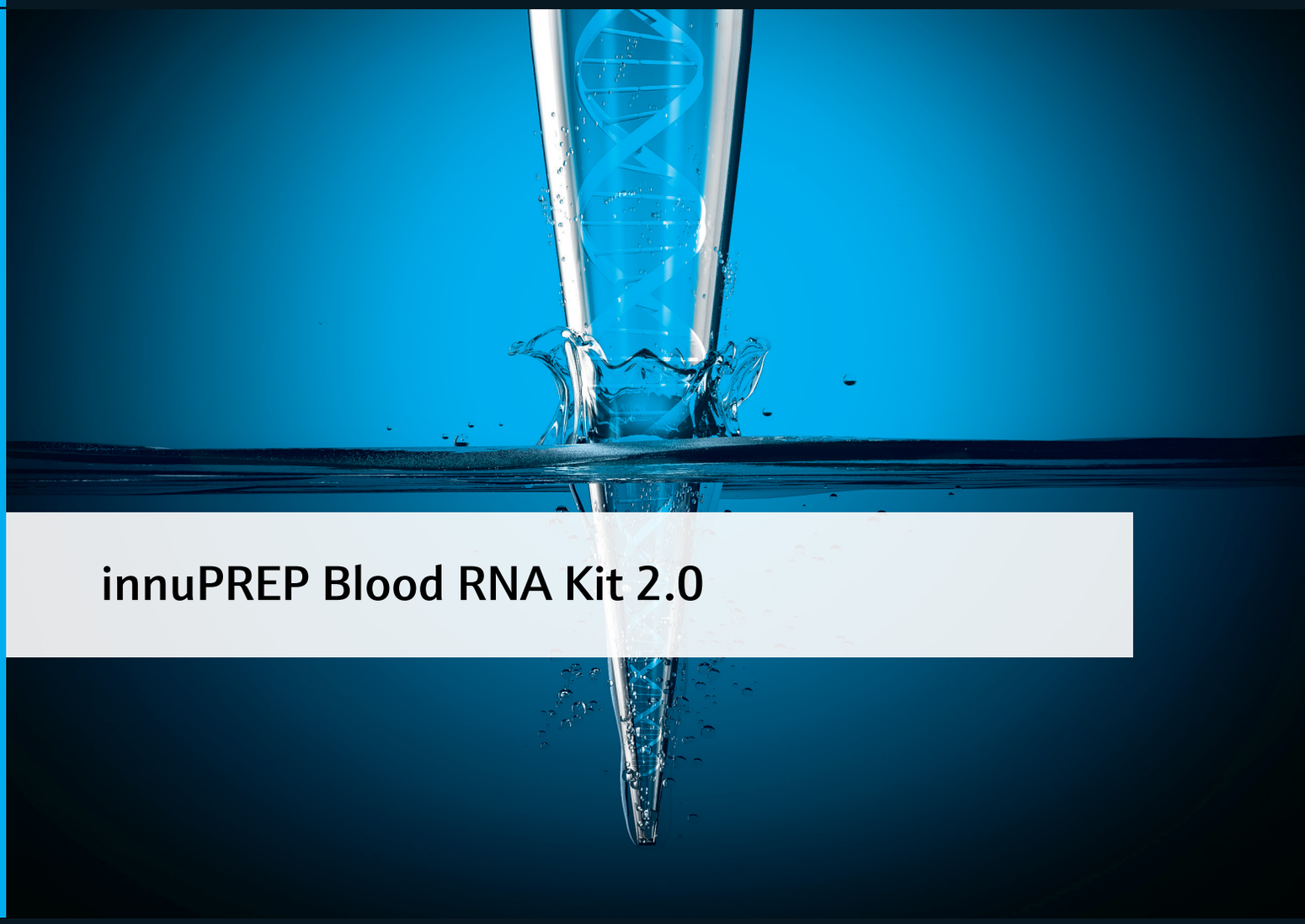
Instructions for Use
Life Science Kits & Assays



innuPREP Blood RNA Kit 2.0

Innuscreen GmbH

Order No.:

845-KS-2010010	10 reactions
845-KS-2010050	50 reactions
845-KS-2010250	250 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

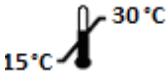
1.1 Intended use

The **innuPREP Blood RNA Kit 2.0** has been designed for the extraction of RNA from fresh whole blood samples. The kit uses an optimized chemistry resulting in a fast and reliable purification of RNA with high quality and 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 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. This kit could be used with potentially 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.

The **innuPREP Blood RNA Kit 2.0** 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 Blood RNA Kit 2.0** 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 our kits have not been validated for any specific application. 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 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	Σ 250
REF	845-KS-2010010	845-KS-2010050	845-KS-2010250
Concentrate ELB	6 ml	30 ml	5 x 30 ml
Lysis Solution RL	8 ml	40 ml	160 ml
Washing Solution MS (conc.)	3 ml	15 ml	60 ml
Washing Solution LS (conc.)	3 ml	16 ml	2 x 40 ml
RNase-free Water	2 ml	6 ml	30 ml
Spin Filter D	10	50	5 x 50
Spin Filter R	10	50	5 x 50
Receiver Tubes	60	6 x 50	30 x 50
Elution Tubes	2 x 10	2 x 50	10 x 50
Manual	1	1	1

6.2 Components not included in the kit

- DNase I; optional
- 15 ml reaction tubes (“Falcon-Tubes”)
- 96–99.8 % and 70 % ethanol (molecular biology grade, non-denatured)
- ddH_2O for diluting Concentrate ELB

7 Product specifications

1. Starting material:
   - Whole blood (0.5–1 ml)
   - Fresh material
   - Stabilizers: EDTA or citrate

2. Time for isolation:
   Approximately 1 hour

3. Binding capacity:
   > 20 µg RNA

4. Typical yield:
   - 1–8 µg
   - Depending on kind and initial volume of whole blood

8 Initial steps before starting

- Ensure that the **Buffer ELB** has been prepared from the **Concentrate ELB** according to the instruction below.
- Add the indicated amount of dd H_2O and Concentrate ELB to an appropriate bottle. Mix thoroughly and always keep the bottle firmly closed.

845-KS-2010010	Dilute 6 ml Concentrate ELB in 194 ml ddH_2O.
845-KS-2010050	Dilute 30 ml Concentrate ELB in 970 ml ddH_2O.
845-KS-2010250	Dilute 30 ml Concentrate ELB in 970 ml ddH_2O.

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

845-KS-2010010	Add 7 ml ethanol to 3 ml Washing Solution MS (conc.).
845-KS-2010050	Add 35 ml ethanol to 15 ml Washing Solution MS (conc.)
845-KS-2010250	Add 140 ml ethanol to 60 ml Washing Solution MS (conc)

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

845-KS-2010010	Add 12 ml ethanol to 3 ml Washing Solution LS (conc.).
845-KS-2010050	Add 64 ml ethanol to 16 ml Washing Solution LS (conc.).
845-KS-2010250	Add 160 ml ethanol to 40 ml Washing Solution LS (conc.)

- Centrifugation steps should be performed at room temperature
- Do not use frozen blood samples.

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 extraction from 0.5 ml up to 1.0 ml of whole blood

IMPORTANT

Please note that up to 1.0 ml of whole blood can be processed.

If the expected number of leukocytes is more than 1 x 10^7, reduce the amount of starting volume of the blood sample.

Do not use frozen blood samples!

1. Transfer **0.5–1.0 ml** of **fresh whole blood** into a 15 ml reaction tube. Add **10 ml Buffer ELB**. Mix shortly by vortexing.
2. Incubate at room temperature for 15 minutes. Mix shortly by vortexing 2 times during incubation. Centrifuge at 2,500 x g for 5 minutes, remove the supernatant completely.

   Don't discard the pellet!
3. Invert the tube on a paper towel and remove the supernatant as complete as possible.

NOTE

Traces of supernatant have an influence on the further purification process.

4. Add **5 ml** of **Buffer ELB** to the cell pellet. Resuspend the cell pellet completely by vigorously shaking (per hand). Centrifuge at 2,500 x g for 3 min, remove the supernatant as far as possible.

   Don't discard the pellet!
5. Invert the tube on a paper towel and remove the supernatant as complete as possible.

NOTE

Traces of supernatant have an influence on the further purification process.

6. Add **600 µl Lysis Solution RL** to the cell pellet. Incubate for 2 minutes at room temperature. Resuspend the cell pellet completely by pipetting up and down.
7. Transfer the resuspended cell pellet into a new 1.5 ml tube and incubate 20 min at 880 rpm.
8. Place a Spin Filter D into a Receiver Tube. Transfer the **lysed sample** onto the Spin Filter D. Centrifuge at 10,000 x g for 2 minutes. Discard the Spin Filter D.

   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.

9. Place a Spin Filter R into a new Receiver Tube, add an **equal volume** (600 µl) of **70 % ethanol** to the filtrate from step 7. Mix the sample by pipetting up and down. Several times. Transfer **650 µl** of the **sample** onto the Spin Filter R. Centrifuge at 10,000 x g 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.

10. Discard the Receiver Tube and place the Spin Filter R into a new Receiver Tube. Load the residual part of the sample onto the same Spin Filter R and centrifuge again at 10,000 x g.

11. Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube.
12. Open the Spin Filter R and add **700 µl Washing Solution MS**, close the cap and centrifuge at 10,000 x g for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
13. Open the Spin Filter R and add **700 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
14. Open the Spin Filter R and add **700 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter R back into the Receiver Tube.
15. Centrifuge at 10,000 x g for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
16. Place the Spin Filter R into an Elution Tube. Carefully open the cap of the Spin Filter R and add **80–100 µl RNase-free Water**. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g for 1 minute.

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 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 contaminations 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 carryover during elution	Increase time for removing of ethanol.
Salt carryover during elution	Ensure that **Washing Solution MS** and **Washing Solution LS** are at room temperature. Checkup Washing Solutions for salt precipitates. If precipitates are present dissolve them by careful warming.

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