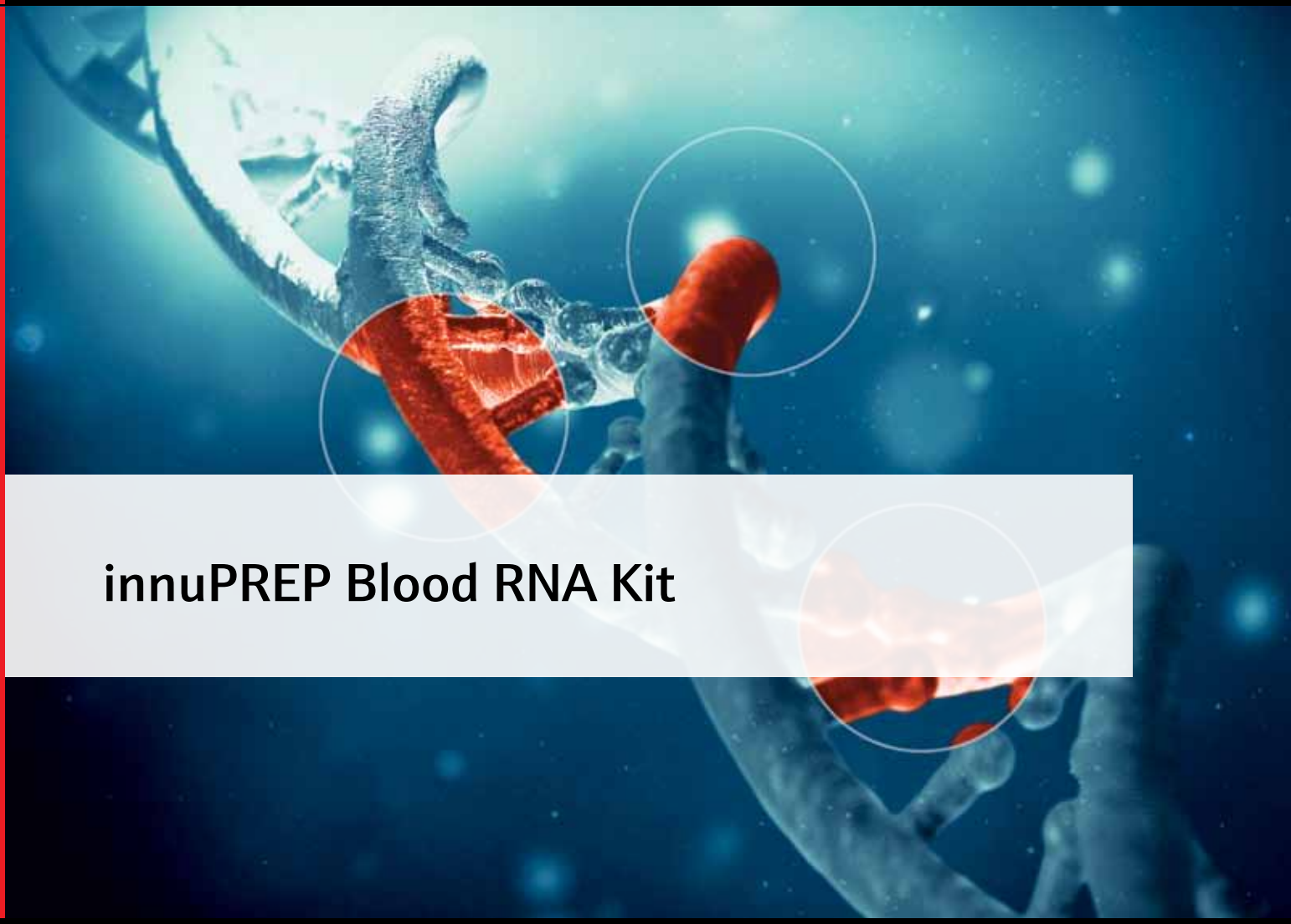
Instructions for Use
Life Science Kits & Assays



innuPREP Blood RNA Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-2010010 10 reactions
845-KS-2010050 50 reactions
845-KS-2010250 250 reactions

Publication No.: HB_KS-2010_e_170713

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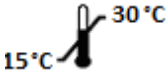
1 Introduction

1.1 Intended use

The innuPREP Blood RNA Kit 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.

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:

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" p. 3).
- Working steps are numbered.

2 Safety precautions

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.

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.

3 Storage conditions

The innuPREP Blood 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. 6).

4 Function testing and technical assistance

The Analytik Jena AG 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 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 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. 9). 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 **Buffer ELB** at 4 °C to 8 °C.
All other components are stored at room temperature (15 °C to 30 °C).

	Σ 10	Σ 50	Σ 250
REF	845-KS-2010010	845-KS-2010050	845-KS-2010250
Buffer ELB	200 ml	-	-
Concentrate ELB	-	30 ml	5 x 30 ml
Lysis Solution RL	8 ml	40 ml	160 ml
Washing Solution HS (conc.)	3 ml	15 ml	70 ml
Washing Solution LS (conc.)	2 ml	8 ml	40 ml
RNase-free Water	2 ml	2 x 2 ml	15 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	10	50	5 x 50
Manual	1	1	1

	Σ 10	Σ 50	Σ 250
Initial steps	**Washing Solution HS (conc.)** Add 3 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!	**Washing Solution HS (conc.)** Add 15 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!	**Washing Solution HS (conc.)** Add 70 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!
	Washing Solution LS (conc.) Add 8 ml of 96 99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!	**Washing Solution LS (conc.)** Add 32 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!	**Washing Solution LS (conc.)** Add 160 ml of 96-99.8 % ethanol to each bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!
		Concentrate ELB Use an appropriate bottle and add 970 ml of ddH_2O and 30 ml Concentrate ELB and mix thoroughly. Keep the bottle always firmly closed.	**Concentrate ELB** Use an appropriate bottle and add 970 ml of ddH_2O and 30 ml Concentrate ELB and mix thoroughly. Keep the bottle always firmly closed.

Components not included in the kit

- DNAse I; optional
- 15 ml reaction tubes (“Falcon-Tubes”)
- 96–99.8 % ethanol

NOTE

Use only absolute/pure ethanol, NO methylated or denatured alcohol!

- ddH_2O
- crushed ice for chilling of components

7 Product specifications

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

2. Time for isolation:
   Approximately 45 minutes

3. Binding capacity:
   > 20 µg RNA

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

8 GHS classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
Lysis Solution RL	Guanidinium thiocyanate 25–50 %	 *Danger*	302, 314, 412	101, 102, 103, 260,303+361+353, 305+351+338, 310, 405, 501	
Concentrate ELB	Ammonium chloride 25–50 %	 *Warning*	319	101, 102, 103, 280, 264, 305+351+338, 403, 501	
Washing Solution HS (conc.)	Guanidinium thiocyanate 50–100 %	 *Danger*	302, 314, 412	101, 102, 103, 260, 310, 405, 501, 303+361+353, 305+351+338,	032

CAUTION
DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

8.1 Hazard phrases

302	Harmful if swallowed.
314	Causes severe skin burns and eye damage.
315	Causes skin irritation.
319	Causes serious eye irritation.
412	Harmful to aquatic life with long lasting effects.

8.2 Precaution phrases

101	If medical advice is needed, have product container or label at hand.
102	Keep out of reach of children.
103	Read label before use.
260	Do not breathe dust/fume/gas/mist/vapors/spray.
264	Wash thoroughly after handling.
310	Immediately call a POISON CENTER/doctor.
403	Store in a well-ventilated place.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
305+351+ 338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

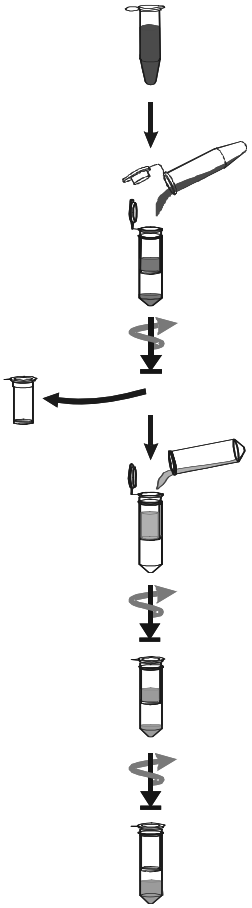
8.3 EU hazard statements

032	Contact with acids liberates very toxic gas

9 Recommended steps before starting

- Ensure that the **Washing Solution HS** and **Washing Solution LS** have been prepared according to the instruction. (→ "Kit components" p. 6)
- Ensure that the **Buffer ELB** has been prepared from the **Concentrate ELB** according to the instruction (→ "Kit components" p. 6). Chill **Buffer ELB** to +4 ˚C before starting the isolation.
- Centrifugation steps should be performed at 4 ˚C to 8 ˚C or room temperature, respectively.
- Don’t use frozen blood samples.

10 General procedure for RNA extraction



Homogenisation / Lysis of starting material

Selective removing of gDNA

Selective binding of RNA

Washing of RNA

Elution of RNA

11 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.

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

Don't use frozen blood samples!

1. Transfer **0.5–1.0 ml** of **fresh whole blood** into a 15 ml reaction tube. Add **10 ml** of cold (+4 °C) **Buffer ELB**. Mix shortly by vortexing.
2. Incubate on ice for 15 minutes. Mix shortly by vortexing 2 times during incubation. Centrifuge at 2,500 x g (3,000 rpm) for 5 minutes at +4 °C, 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. Re-suspend the cell pellet completely by vigorously shaking (per hand). Centrifuge at 2,500 x g (3,000 rpm) for 3 min at +4 °C, 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. Re-suspend the cell pellet completely by pipetting up and down.

NOTE

To maximize the final yield of total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step. If necessary incubate another 2 minutes at room temperature.

7. Place a Spin Filter D into a Receiver Tube. Transfer the **lysed sample** onto the Spin Filter D. Centrifuge at 10,000 x g (12,000 rpm) 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.

8. 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 sometimes up and down. Transfer **650 µl** of the **sample** onto the Spin Filter R. 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.

9. 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 (12,000 rpm).
10. Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube.
11. Open the Spin Filter R and add **500 µl Washing Solution HS**, 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 R into a new Receiver Tube.
12. Open the Spin Filter R and add **700 µl Washing Solution LS**, 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 R into a new Receiver Tube.
13. Centrifuge at 10,000 x g (12,000 rpm) for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
14. Place the Spin Filter R into an Elution Tube. Carefully open the cap of the Spin Filter R and add **30–50 µl RNase-free Water**. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (8,000 rpm) for 1 minute.

NOTE

Depending on the extracted yield or the needed concentration of total RNA you can also elute with different volumes of RNase-free water. A lower volume of RNase-free Water increases the concentration of RNA and a higher volume of RNase-free Water leads to an increased yield but a lower concentration of total RNA. Please note, that the minimum of RNase-free Water should be 20 µl.

13 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 a 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 HS** and **Washing Solution LS** are at room temperature. Check up Washing Solutions for salt precipitates. If there are any precipitates dissolve these precipitates by careful warming.

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Subject to changes in design and scope of delivery as well as further technical development!