

Instructions for Use

Life Science Kits & Assays



innuPREP RNA Mini Kit 2.0

Order No.:

845-KS-2040010 10 reactions
845-KS-2040050 50 reactions
845-KS-2040250 250 reactions

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1 Introduction

1.1 Intended use

The innuPREP RNA Mini Kit 2.0 has been designed for simple, reliable and fast isolation of total RNA. The kit can be used for isolation of RNA from tissue samples, eukaryotic cells and Gram-negative or Gram-positive bacteria. The isolation procedure is based on a new kind of patented technology.



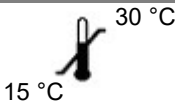







CONSULT INSTRUCTION FOR USE

This package insert must be read carefully prior to 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. → „

CONSULT INSTRUCTION FOR USE

This package insert must be read carefully prior to 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.

- Notes on the use of this manual“ p. 3).
- Working steps are numbered.

2 Safety precautions

NOTE

Read through this chapter carefully prior 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.

The innuPREP RNA Mini 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.

For further information see chapter "Kit components" (→ p. 8).

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 RNA Mini 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 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 (→ "Intended use" p. 2), (→ "Product specifications" p. 10). 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

	 10	 50	 250
REF	845-KS-2040010	845-KS-2040050	845-KS-2040250
Lysis Solution RL	6 ml	30 ml	125 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	6 ml	2 x 15 ml
Spin Filter D	10	50	5 x 50
Spin Filter R	10	50	5 x 50
Receiver Tubes	50	5 x 50	25 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1

6.2 Components not included in the kit

- 1.5 ml reaction tubes
- Ethanol (70 %, 96–99.8 %); non denatured or methylated
- ddH₂O

Components needed for isolation of RNA from tissue samples (optional)

- innuSPEED Lysis Tube P
- DNase I
- TE-Buffer (10 mM Tris-HCl; 1 mM EDTA; pH 8.0)
- 80 % ethanol; non denatured or methylated

Components needed for isolation of RNA from bacteria (optional)

- Lysozyme (stock solution: 10 mg/ml, 400 U/ μ l)
- Mutanolysin (stock solution: 0.4 U/ μ l)
- Lysostaphin (stock solution: 0.4 U/ μ l)
- TE-Buffer (10 mM Tris-HCl; 1 mM EDTA; pH 8.0)
- Alternatively: innuPREP Bacteria Lysis Booster

7 Product specifications

1. Starting material:
 - Eukaryotic cells (up to 5×10^6 cells)
 - Tissue samples (up to 20 mg)
 - Gram-positive and Gram-negative bacteria (up to 1×10^9 cells)
 - Biopsies (up to 20 mg)

NOTE

Avoid freezing and thawing of starting material.

2. Time for isolation:
 - Approximately 15–40 minutes
3. Typical yield:
 - Depends on the type and the amount of the starting material
4. Binding capacity:
 - Approximately 100 μg RNA

8 Initial steps before starting

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

845-KS-2040010	Add 3 ml ethanol to 3 ml Washing Solution HS (conc.).
845-KS-2040050	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
845-KS-2040250	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).

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

845-KS-2040010	Add 8 ml ethanol to 2 ml Washing Solution LS (conc.).
845-KS-2040050	Add 32 ml ethanol to 8 ml Washing Solution LS (conc.).
845-KS-2040250	Add 160 ml ethanol to 40 ml Washing Solution LS (conc.).

- 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 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 ddH₂O.
- 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 1: RNA extraction from tissue samples

IMPORTANT

Please note that up to 20 mg of tissue samples can be processed.
Avoid freezing and thawing of tissue samples!

1. Homogenization of starting material.
-

NOTE

To maximize the final yield of total RNA a complete homogenization of tissue sample is important!

For the homogenization of tissue samples it is possible to use commercially available rotor-stator homogenizer or bead mills. It is also possible to disrupt the starting material using mortar and pestle in liquid nitrogen and grind the tissue sample to a fine powder.

- A. **Homogenization of tissue samples using a rotor-stator homogenizer**
 1. Transfer the weighed amount of fresh or frozen starting material in a suitable reaction vessel for the homogenizer.
 2. Add 450 μ l Lysis Solution RL.
 3. Homogenize the sample.
 4. Transfer the homogenized tissue sample into a 1.5 ml reaction tube and place the sample in Lysis Solution RL for longer storage at -22 °C to -18 °C or use the sample immediately for isolation of total RNA following the protocol step 2.

B. Disruption of the tissue sample using a mortar and pestle and liquid nitrogen

1. Transfer the weighed amount of fresh or frozen starting material under liquid nitrogen and grind the material to a fine tissue powder.
 2. Transfer the powder into a 1.5 ml reaction tube. Don't allow the sample to thaw!
 3. Add **450 µl Lysis Solution RL** and incubate the sample for appropriate time for a further lysis under continuous shaking.
 4. Finally place the sample under **Lysis Solution RL** for longer storage at -22 °C to -18 °C or use the sample immediately for isolation of total RNA following protocol step 2.
-
2. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute. Place a Spin Filter D into a Receiver Tube. Transfer the supernatant of the lysed sample onto the Spin Filter D. Centrifuge at 11,000 x g (~11,000 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.

3. Place a Spin Filter R into a new Receiver Tube. Add an **equal volume** (approx. 400 µl) of **70 % ethanol** to the filtrate from step 2. Mix the sample by pipetting up and down several times. Transfer the sample onto the Spin Filter R. Centrifuge at 11,000 x g (~11,000 rpm) 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.

Protocol 1: RNA extraction from tissue samples

Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube.

4. Open the Spin Filter R and add **500 µl Washing Solution HS**, close the cap and centrifuge at $11,000 \times g$ (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
5. Open the Spin Filter R and add **700 µl Washing Solution LS**, close the cap and centrifuge at $11,000 \times g$ (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.

NOTE

Depending on sample material used an additional washing step with **700 µl of 80 % ethanol** may increase purity of isolated RNA.

6. Centrifuge at $11,000 \times g$ (~11,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
7. Place the Spin Filter R into an Elution Tube. Carefully open the cap of the Spin Filter R and add **30–80 µl RNase-free Water**. Incubate at room temperature for 1 minute. Centrifuge at $11,000 \times g$ (~11,000 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.

11 Protocol 2: RNA extraction from eukaryotic cells

IMPORTANT

Please note that up to 5×10^6 cells can be processed.

1. Add **400 μ l 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. Incubate the sample for further 3 minutes at room temperature.
-

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.

2. Place a Spin Filter D into a Receiver Tube. Transfer the lysed sample onto the Spin Filter D. Centrifuge at $11,000 \times g$ (~11,000 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.

3. Place a Spin Filter R into a new Receiver Tube. Add an **equal volume** (approx. 400 μ l) of **70 % ethanol** to the filtrate from step 2. Mix the sample by pipetting several times up and down. Transfer sample onto the Spin Filter R. Centrifuge at $11,000 \times g$ (~11,000 rpm) 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.

Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube.

4. Open the Spin Filter R and add **500 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
 5. Open the Spin Filter R and add **700 µl Washing Solution LS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
-

NOTE

Depending on sample material used an additional washing step with **700 µl of 80 % ethanol** may increase purity of isolated RNA.

6. Centrifuge at 11,000 x g (~11,000 rpm) for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
 7. Place the Spin Filter R into an Elution Tube. Carefully open the cap of the Spin Filter R and add **30–80 µl RNase-free Water**. Incubate at room temperature for 1 minute. Centrifuge at 11,000 x g (~11,000 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.

12 Protocol 3: RNA extraction from bacterial cells

IMPORTANT

Please note that up to 1×10^9 cells can be processed.

We recommend a pre-incubation of bacterial cells with Lysozyme or optionally available **innuPREP Bacteria Lysis Booster**.

12.1 Collection of bacterial cells

1. Transfer the bacterial culture (volume depends on the concentration of starting material) into a tube 2.0 ml tube.
2. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes at 3,000 x g). Discard the supernatant.

Do not discard the pellet!

3. Resuspend the bacterial cell pellet in **100 µl TE-Buffer**.
4. Proceed with appropriate pre-lysis step.

12.2 Pre-lysis of Gram-negative bacteria

NOTE

Although Gram-negative bacteria do not require a pre-lysis step, using Lysozyme (not included in the kit) can enhance the efficiency of lysis.

1. Add **20 µl Lysozyme** (10 mg/ml). Pipette several times up and down; the solution should become clear or viscous.
2. Proceed with **"Isolation of bacterial RNA"** on p. 22.

12.3 Pre-lysis of Gram-positive bacteria

NOTE

Gram-positive bacteria require a pre-lysis step using Mutanolysin and/or Lysozyme (not included in the kit). As both exert synergistic activity a simultan usage will increase the yield of isolated nucleic acids.

The optionally available **innuPREP Bacteria Lysis Booster** contains all enzymes necessary for highly efficient lysis of bacteria (→ p. 21).

1. Add **20 µl Lysozyme** (10 mg/ml) and/or **5 µl Mutanolysin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with "Isolation of bacterial RNA" on p. 22.

12.4 Pre-lysis of *Staphylococcus* strains

NOTE

For pre-lysis of *Staphylococcus* the enzyme Lysostaphin is recommended (not included in the kit).

The optionally available **innuPREP Bacteria Lysis Booster** contains all enzymes necessary for highly efficient lysis of bacteria (→ p. 21).

1. Add **10 µl Lysostaphin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
2. Proceed with "Isolation of bacterial RNA" on p. 22.

12.5 Pre-lysis of bacteria using optional innuPREP Bacteria Lysis Booster

NOTE

The innuPREP Bacteria Lysis Booster Kit has been developed for a highly efficient pre-lysis of bacterial cell walls by generating spheroblasts. This new mixture of different enzymes boost the lysis of all bacteria in particular the hard-to-lyse microorganisms like *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Bacillus* and *Clostridium*.

1. Transfer the bacterial culture (volume depends on the concentration of starting material) into a tube 2.0 ml tube.
2. Collect the cells by centrifugation with parameters adequate for the cell type (e.g. 10 minutes at 3,000 x g). Discard the supernatant.

Do not discard the pellet!
3. Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.
4. Resuspend the bacterial cell pellet in **170 µl TE-Buffer**.
5. Add **20 µl Enzyme Mix** to the sample and vortex shortly. Incubate the sample for 30 minutes at 37 °C.
6. Proceed with "Isolation of bacterial RNA" on p. 22.

12.6 Isolation of bacterial RNA

NOTE

The following protocol is done after pre-lysis of samples.

1. Add **450 µl Lysis Solution RL** to the pre-lysed sample and vortex vigorously or pipette sometimes up and down. Incubate the sample for further 3 minutes at room temperature.
-

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.

2. Place a Spin Filter D into a Receiver Tube. Transfer the lysed sample onto the Spin Filter D. Centrifuge at 11,000 × g (~11,000 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.

3. Place a Spin Filter R into a new Receiver Tube. Add an **equal volume** (approx. 600 µl) of **70 % ethanol** to the filtrate from step 2. Mix the sample by pipetting several times up and down.

4. Transfer **650 µl** of the **sample** onto the Spin Filter R. Centrifuge at **11,000 x g (~11,000 rpm)** for 1 minute. Discard the Receiver Tube and place the Spin Filter R into a new Receiver Tube. **Load the residual sample** on the Spin Filter R and centrifuge again at **11,000 x g (~11,000 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.

Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube.

5. Open the Spin Filter R and add **500 µl Washing Solution HS**, close the cap and centrifuge at **11,000 x g (~11,000 rpm)** for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
 6. Open the Spin Filter R and add **700 µl Washing Solution LS**, close the cap and centrifuge at **11,000 x g (~11,000 rpm)** for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
-

NOTE

Depending on sample material used an additional washing step with **700 µl of 80 % ethanol** may increase purity of isolated RNA.

7. Centrifuge at **11,000 x g (~11,000 rpm)** for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube.
8. Place the Spin Filter R into an Elution Tube. Carefully open the cap of the Spin Filter R and add **30–80 µl RNase-free Water**. Incubate at room temperature for 1 minute. Centrifuge at **11,000 x g (~11,000 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 μ l.

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 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, has 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 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. Checkup Washing Solution for salt precipitates. If there are any precipitate dissolves these precipitate by carefully warming.

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