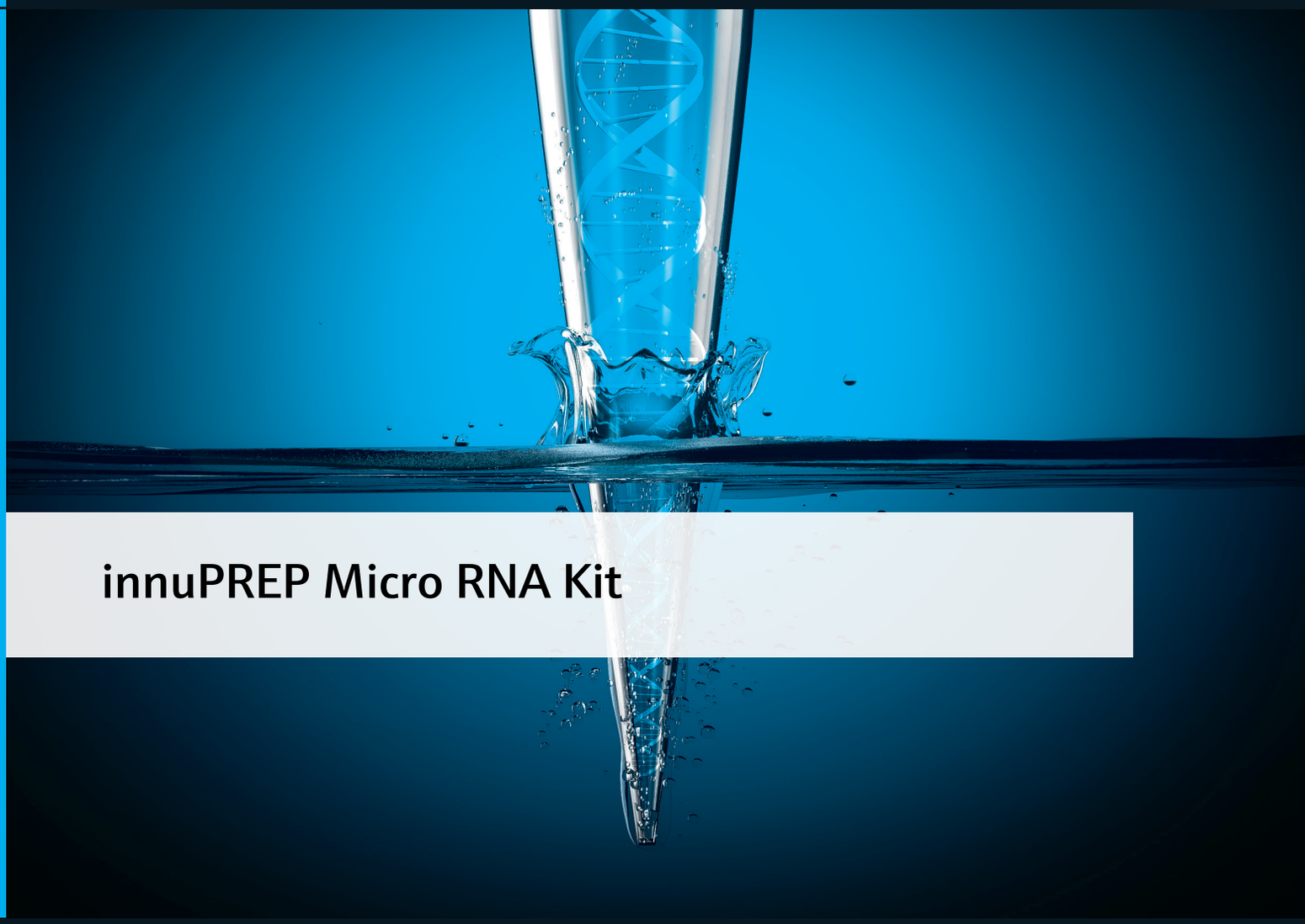
Instructions for Use
Life Science Kits & Assays



innuPREP Micro RNA Kit

Innuscreen GmbH

Order No.:

845-KS-2030010 10 reactions
845-KS-2030050 50 reactions
845-KS-2030250 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 Micro RNA Kit has been designed for the extraction of small RNA molecules from eukaryotic cells, tissue samples, biopsies and bacterial cells. 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 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.

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. 2).
- 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. 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 nu-

cleic 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 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.

4 Storage conditions

The Kit is shipped at ambient temperature and stored at room temperature (15 °C to 30 °C) upon arrival. Under these conditions 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.

5 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 Micro 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.

6 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.

7 Kit components

7.1 Included kit components

	Σ 10	Σ 50	Σ 250
REF	845-KS-2030010	845-KS-2030050	845-KS-2030250
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	25 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

7.2 Components not included in the kit

- 1.5 ml reaction tubes
- ≥ 99.8 % 2-Propanol
- 96–99.8 % ethanol, non denatured or methylated

For Protocol 3:

- TE-Buffer (10 mM Tris-HCl; 1 mM EDTA; pH 8.0)
- Lysozyme (10 mg/ml, 400 U/µl), Mutanolysin (0.4 U/µl), Lysostaphin (0.4 U/µl) **OR** innuPREP Bacteria Lysis Booster (IST Innuscreen GmbH, 845-KA-1000050)

8 Product specifications

1. **Starting material**

- up to 5 x 10^6 eucaryrotic cells
- up to 20 mg tissue samples
- up to 1 x 10^9 Gram-positive and Gram-negative bacteria
- Biopsies

2. **Time for isolation**

- Approximately 15 – 40 minutes after lysis step

3. **Typical yield**

- Depends on the type and amount of starting material
- Binding capacity of Spin Filter columns approx. 100 µg RNA

9 Recommended steps before starting

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

845-KS-2030010	Add 3 ml ethanol to 3 ml Washing Solution HS (conc.).
845-KS-2030050	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
845-KS-2030250	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.
  Always keep the bottle firmly closed.

845-KS-2030010	Add 8 ml ethanol to 2 ml Washing Solution LS (conc.).
845-KS-2030050	Add 32 ml ethanol to 8 ml Washing Solution LS (conc.).
845-KS-2030250	Add 160 ml ethanol to 40 ml Washing Solution LS (conc.).

- Centrifugation steps Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

10 Protocol 1: RNA extraction from tissue samples

10.1 Homogenization of starting material

For the homogenization of tissue sample 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.

NOTE

To maximize the final yield of total RNA a complete homogenization of tissue sample is important! Avoid freezing and thawing of the sample.

A Homogenization using a rotor-stator homogenizer or bead mills

1. Weigh up to 20 mg of fresh or frozen starting material (tissue, biopsie) and transfer it in a suitable reaction vessel for the homogenizer or in a vessel pre-filled with beads.
2. Add **450 µl Lysis Solution RL.**
3. Homogenize the sample.
4. Transfer the homogenized tissue sample into a 1.5 ml reaction tube and 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 the protocol step 2.

B Disruption using a mortar and pestle

1. Weigh up to 20 mg fresh or frozen starting material (tissue, biopsie) in a pre-cooled mortar. Grind the material under liquid nitrogen with a pestle to a fine 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.

10.2 RNA extraction from tissue samples

1. Spin down unlysed material by centrifugation at full 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 10,000 x g (~12,000 rpm) for 2 minutes. Discard the Spin Filter D.

ATTENTION!
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 a Spin Filter R into a new Receiver Tube. Add an **equal volume** (appr. 400 µl) of **2-Propanol** to the filtrate from step 4. Mix the sample by pipetting up and down several times. Transfer the sample onto the Spin Filter R. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.

NOTE
To remove DNA from sample (optional) perform an on-column DNase digest.

3. 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 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.
5. 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.
6. Centrifuge at full speed 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 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.

11 Protocol 2: RNA extraction from eucaryotic cells

1. Transfer up to 5 x 10^6 eukaryotic cells into a tube 2.0 ml tube and pellet the cells by centrifugation with parameters adequate for the cell type (e.g. 2-5 minutes at 5,000 x g). Discard the supernatant. Do not discard the pellet!

2. Add **400 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. 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.

3. 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.

ATTENTION!
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.

4. Place a Spin Filter R into a new Receiver Tube. Add an **equal volume** (appr. 400 µl) of **2-Propanol** to the filtrate from step 2. Mix the sample by pipetting sometimes up and down. Transfer sample onto the Spin Filter R. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.
5. Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube.
6. 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.
7. 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.
8. Centrifuge at full speed for 3 minutes to remove all traces of ethanol. Discard the Receiver Tube
9. 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 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.

12 Protocol 3: RNA extraction from bacterial cells

12.1 Pre-lysis of bacterial cell walls

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. 2-5 minutes at 5,000 x g (~7.500 rpm)). Discard the supernatant. Do not discard the pellet!
3. Resuspend the bacterial cell pellet in **100 µl TE-Buffer**.

12.1.1 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.

4. Add **20 µl Lysozyme** (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
5. Proceed with “Isolation of bacterial RNA” on p. 14.

12.1.2 Gram-positive bacteria

NOTE
Gram-positive bacteria require a pre-lysis step using Mutanolysin and/or Lysozyme (not included in the kit).

4. Add **20 µl Lysozyme** (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
5. Add **5 µl Mutanolysin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
6. Proceed with “Isolation of bacterial RNA” on p. 14.

NOTE
Lysozyme and Mutanolysin exert synergistic activity. Using both enzymes together will increase the yield of isolated nucleic acids.

12.1.3 Staphylococcus strains

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

4. Add **10 µl Lysostaphin** (0.4 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking.
5. Proceed with "Isolation of bacterial RNA" on p. 14.

12.1.4 Alternative pre-lysis using 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*.

4. Add **70 µl TE-Buffer** to the resuspended cell pellet and mix again.
5. Prepare the enzyme mix according to the manual of the innuPREP Bacteria Lysis Booster.
6. Add **12 µl enzyme mix** to the sample and vortex shortly. Incubate the sample for 30 minutes at 37 °C.
7. Proceed with "Isolation of bacterial RNA" on p. 14.

12.2 Isolation of bacterial RNA

1. Add **450 µl Lysis Solution RL** to the 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.

2. 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.

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

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.

3. Place a Spin Filter R into a new Receiver Tube. Add an **equal volume** (appr. 600 µl) of **2-Propanol** 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 10,000 x g (~12,000 rpm) for 2 minutes.
4. 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 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.
6. 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.
7. Centrifuge at full speed 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 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	Increase lysis time! Increase centrifugation speed! After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.
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 Elution Buffer.
Insufficient mixing with 2-Propanol	Mix sample with 2-Propanol by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
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 an on-column digest
Total RNA degraded	
Incorrect storage of starting material	Ensure that the starting material is fresh (long time storage at -78 °C to -82° C) Avoid thawing of the material. Ensure that the first steps of the protocol are performed quickly!
Old starting material	Old material often contains degraded DNA. Repeat with fresh material.
RNase contamination of solutions	Use sterile, RNase-free filter tips. Before every preparation clean up pipette, devices and working place. Always wear gloves.
Total RNA does not perform well in downstream applications	
Ethanol carryover during elution	Increase time for removing ethanol.
Salt carryover during elution	Ensure that Washing Solution HS and Washing Solution LS have room temperature. Check the solutions for salt precipitates. If there are any dissolve these by carefully warming.

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