Instructions for use

ROTI®Prep RNA MINI

8485

For RNA isolation from eukaryotic cells, tissues, bacteria, biopsies

Introduction and product description:

- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Use of DNase not necessary
- Preparation in approx. 15 to 40 minutes
- Yield up to 100 µg RNA, depending on source material
- Elution in 30-80 µl

ROTI®Prep RNA MINI has been developed for the isolation of total RNA from various sources. The isolation is carried out through the proven and reliable spin column technique, which is very easy to handle and needs only few steps.

One of the critical points in RNA extraction is the presence of endogenous RNases, which can lead to high losses very early in extraction of the (in comparison to DNA) very sensitive RNA. Therefore the lysis buffer has been specially optimised to inactivate RNases, with highest efficiency, already at the early stage of the extraction.

Furthermore, in order to get a high-pure total RNA, one particularly for this purpose developed prefilter is used. This filter exclusively serves for binding and targeted removal of DNA impurities, and is discarded after use with the bound DNA. Thus, it is possible to omit application of DNase I. Total RNA is then obtained from the DNA-free eluate through a simple "Binding-Washing-Elution" process.

In each preparation, up to 100 µg highly pure total RNA may be isolated. One preparation takes (excluding lysis) approx. 10 mins. Isolated RNA is free of DNA and enzyme inhibitors, and may directly be applied to RT-PCR or other downstream applications.

Suitable source material:

- Tissue (up to 20 mg)
- Eukaryotic cells (up to $5x10^6$ cells)
- Yeast cells (up to $5x10^7$ cells)
- Bacteria (Gram+ and Gram -)
- Biopsies

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Cautions:

*Lysis Buffer LSR** **Danger** H302+312+H332-H314-H412-EUH032

Washing Buffer WSA **Danger** H302+312+H332-H314-H412-EUH032

*In case of forming of precipitates, please dissolve by careful warming.

MSDS: the appropriate MSDS can be downloaded from our website www.carlroth.com.

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.

Literature Citation: When describing a procedure for publication using this product, please refer to it as *Carl Roth's ROTI®Prep RNA MINI Kit*.

1. Materials provided with the kit and storage conditions

Amount	Component	Storage
15 / 30 / 160 ml	Lysis Buffer LSR*	>20 °C**
2 / 3x2 / 25 ml	RNase-free water	RT
5 / 15 / 70 ml	Washing Buffer WSA (Base)	RT
6 / 16 / 36 ml	Washing Solution WSL (Base)	RT
10 / 50 / 250	Mini spin columns (blue)	RT
10 / 50 / 250	Mini spin columns (violet)	RT
10 / 50 / 250	1.5 ml Elution tubes	RT
50 / 250 / 1250	2 ml Collection tubes	RT

* The Lysis Buffer LSR is now filled in a white bottle.

** Lysis Buffer should be stored at over 20 °C in order to prevent precipitation of reagents.

The ROTI®Prep RNA MINI Kit should be stored dry, at room temperature (14-25 °C). Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming. This kit is stable for at least 6 months after receipt, when stored as directed.

Contents of this Kit may not be bought separately.

The components of each ROTI®Prep RNA MINI Kit were tested in general by isolation of total RNA from tissue samples and subsequent analysis on an Agilent Bioanalyzer. The user is responsible, however, to validate the performance of the ROTI®Prep RNA MINI Kit for isolation of RNA from his particular samples, since the performance characteristics of our kits have not been validated for any specific application.

2. Required Material and Equipment not included in this kit

- Ethanol 96-99.8 %, e.g. Ethanol p.a. (e.g. 9065.1)
- DNAse I (optional)
- Lysozyme (e.g. 8259.1) (optional, for isolation from bacteria)
- Sorbitol buffer (optional, for isolation from yeast cells)
  1.2 M sorbitol, 0.1 M sodium citrate, pH 7.6, 0.06 M EDTA (similar comp. may be used)
- Distilled, sterilized H_2O, (e.g. 3255.1)
- Distilled, sterilized H_2O, RNase-free, for preparation of 70 % ethanol, (e.g. T143.1)
- TE buffer (e.g. ROTI®Stock 10x TE, 1052.1)
- DEPC (e.g. K028.1)

3. Application

ROTI®Prep-RNA MINI is designed for isolation of high-purity RNA from various source materials. Spin column based preparation allows elution in a small volume of low-salt buffer, eliminating time-consuming phenol-chloroform extraction or alcohol precipitation. The columns may be used either in micro-centrifuges or on vacuum manifolds.

The kit allows the extraction of **up to 100 µg** RNA per preparation from up to 20 mg of tissue, biopsies, bacteria, or cultured cells (up to $5x10^6$).

As in all isolation procedures, lysis is a crucial step and has to be performed very carefully. Complete lysis of the sample is vital for a good recovery rate of RNA. However, RNA is highly temperature sensitive and has to be kept cool as soon as possible. It is important, therefore, to prolong all steps as long as necessary, but also as short as possible.

The homogenised sample may be stored in Lysis Buffer LSR at -80 °C. Short term storage may be done at -20 °C.

Repetition of elution (final step) may the recovery rate overall. However, even so often, concentration of the recovered RNA is reduced in parallel, since the whole amount of water used for elution is higher.

Store the extracted RNA at -80 °C. Avoid repeated freeze & thaw cycles by all means.

For centrifugation we recommend a standard microcentrifuge.
Centrifugation steps should be carried out at room temperature.

Avoid repeated freeze&thaw cycles for the tissue to be extracted.

4. General Comments on Handling of 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. In order 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:

- Clear the bench top first using RNase AWAY® (A998.1) or ROTI®Nucleic Acid-free (HP69.1).
- Always wear latex or vinyl gloves while handling reagents and RNA samples in order to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- **Autoclaving will not inactivate RNases.**
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with RNase AWAY® (A998.1) or ROTI®Nucleic Acid-free (HP69.1), followed by RNase-free water.
- All glassware should be treated before use in order to ensure that it is RNase-free. Glassware should be cleaned with RNase AWAY® (A998.1) or ROTI®Nucleic Acid-free (HP69.1), thoroughly rinsed with RNase-free water, and oven baked at 240 °C for four or more hours before use. Oven baking in-activates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware.
- Reduce preparation time as much as possible.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Electrophoresis tanks should be cleaned with RNase AWAY® (A998.1) or ROTI®Nucleic Acid-free (HP69.1), followed by RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free double-distilled water.
- 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.

Before start, be sure to

- Add Ethanol (96-99.8 %, not incl. in this kit) to the Washing Buffer WSA as follows:
  8485.1 (10 Preps): 5 ml (10 ml final vol.)
  8485.2 (50 Preps): 15 ml (30 ml final vol.)
  8485.3 (250 Preps): 70 ml (140 ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!
- Add Ethanol (96-99.8 %, not incl. in this kit) to the Washing Solution WSL as follows:
  8485.1 (10 Preps): 24 ml (30 ml final vol.)
  8485.2 (50 Preps): 64 ml (80 ml final vol.)
  8485.3 (250 Preps): 144 ml (180 ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!
- Prepare 70 % ethanol (RNase-free) by mixing 70 ml ethanol (above) with 30 m RNase-free water. Use RNase-free glass-ware.
- For isolation of RNA from bacteria:
  Prepare a stock solution of lysozyme.
  For gram- bacteria: 20 mg / ml in water
  For gram+ bacteria: 50 mg / ml in water
  Store lysozyme in aliquots at -20 °C

Prepare 1x TE buffer by diluting 1 ml ROTI®Stock 100 x TE with 99 ml distilled, sterile water.

Please note: Centrifugation steps should be carried out at room temperature

5. Workflow

The workflow has been provided on a separate page, in order to use a copy for in-process protocol or filing in your personal lab files.

5.1 RNA isolation from tissue samples (max. 20 mg tissue)

Please note: In order to maximize the final yield of total RNA complete homogenization of tissue sample is important.
We recommend using a rotor-stator homogenizer or a bead mill for homogenization of tissue sample. 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.

Step (RT = room temperature)	**done**

1a. Tissue lysis with a homogeniser	
Place max. 20 mg of the tissue sample (fresh or frozen material) into a suitable vessel for homogenisation.	
Add 450 µl **Lysis Buffer LSR**, and homogenise the sample to fine powder according to instructions accompanying the instrument used.	
Transfer the homogenised sample to a 1.5 ml reaction tube.*	

1b.Tissue lysis with mortar and pestle in liquid nitrogen	
Place max. 20 mg of the tissue sample (fresh or frozen material) into a suitable vessel for grinding (e.g. a mortar). When using fresh material: add liquid nitrogen for freezing.	
Grind the sample with a pestle to fine powder.	
Transfer the homogenised sample to a 1.5 ml reaction tube. Don't allow the sample to thaw!	
Add 450 µl **Lysis Buffer LSR** *, and incubate the sample for 30 mins. at RT under continuous shaking.**	

2. Removal of DNA / First Column Loading	
Precipitate unlysed material by centrifugation at max. speed for 1 min/RT.	
Place a **blue** Spin Column into a 2 ml collection tube.	
Apply the supernatant to the blue spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT. **Keep the filtrate containing the RNA** and discard the spin filter.***	

3. Binding of RNA / Second Column Loading	
Place a **violet** Spin Column into a new 2 ml collection tube.	
Add an equal volume (approx. 400 µl) of 70 % ethanol to the filtrate from step 2. Mix by pipetting up and down.	
Apply the mix of filtrate/ethanol to the violet spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT and discard the flow-through.****	

Sequel: RNA isolation from tissue samples

Step (RT = room temperature)	**done**

4. Column Washing	
Place the violet Spin Column into a new collection tube.	
Add 500 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through****	
Add 700 µl of **Washing Solution WSL** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through****	
Again centrifuge columns at 10.000 g (or 12.000 rpm) for 3 mins/RT in order to remove residual ethanol.	

5. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50 µl **RNase-free water** (incl. in the kit) to the centre of the membrane*****	
Incubate for 1 min at room temperature	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute RNA	

* The homogenised sample may be stored in Lysis Buffer LSR at -80 °C. Short term storage may be done
at -20 °C.
** recommend to incubate for 30 mins in a first approach, the appropriate time span for further lysis, however, depends on the material and has to be optimized. Check lysis efficiency before proceeding in the protocol. If the lysis is not complete, prolong the incubation time in Lysis Buffer LSR. This step is critical, don't be impatient. We recommend using a shaking platform or thermomixer for continuous agitation of the sample. Alternatively, vortex the sample 3-4 times during incubation. Agitation is vital for a decent recovery rate.
*** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time. The Spin Filter contains the DNA and is discarded.
Do NOT discard the supernatant containing the RNA!
**** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.
***** Elution with lower volumes of water (e.g. 20-30 µl) increases the final concentration of RNA. Higher volumes (80-100 µl) result in lower concentration of RNA, although the overall amount of RNA eluted may be lightly higher. Minimum elution amount is 20 µl.

5.2 RNA isolation from eukaryotic cells (max. $5x10^6$ cells)

Adherent cells should be loosened from the culture bottle surface by trypsination or EDTA. Remove cells and wash in PBS. Count your cell number and select the appropriate cell amount, $5x10^6$ cells in maximum.

Step (RT = room temperature)	**done**

1. Cell lysis	
Pellet cells by centrifugation at 5.000 g (or 7.500 rpm) for 10 mins and discard the supernatant.*	
Add 400 µl **Lysis Buffer LSR** and incubate for 2 mins/RT.	
Resuspend cells completely by pipetting up and down. Incubate further (approx. 3 mins) at RT until the sample is *completely* lysed.**	

2. Removal of DNA / First Column Loading	
Place a **blue** Spin Column into a 2 ml collection tube.	
Apply the lysed cell solution to the blue spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT. **Keep the filtrate containing the RNA** and discard the spin filter.***	

3. Binding of RNA / Second Column Loading	
Place a **violet** Spin Column into a new 2 ml collection tube.	
Add an equal volume (approx. 400 µl) of 70 % ethanol to the filtrate from step 2. Mix by pipetting up and down.	
Apply the mix of filtrate/ethanol to the violet spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT and discard the flow-through.****	

4. Column Washing	
Place the violet Spin Column into a new collection tube.	
Add 500 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through****	
Add 700 µl of **Washing Solution WSL** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through****	
Again centrifuge columns at 10.000 g (or 12.000 rpm) for 3 mins/RT in order to remove residual ethanol.	

5. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50 µl **RNase-free water** (incl. in the kit) to the centre of the membrane*****	
Incubate for 1 min at room temperature	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute RNA	

* Alternatively, adherent cells may be incubated in 96wells. Wash cells with PBS and perform the lysis in the 96well under agitation. Following lysis, the cell solution has to be transferred into 1.5 ml tubes for centrifugation.

** The appropriate time span for further lysis has to be optimized. Check lysis efficiency before proceeding in the protocol. If the lysis is not complete, prolong the incubation time in Lysis Buffer LSR. **No cell clumps should be visible after this step!** This step is critical, don't be impatient. The lysed cells may be stored in Lysis Buffer LSR at -80 °C. Short term storage may be done at -20 °C.

*** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time. The Spin Filter contains the DNA and is discarded. **Do NOT discard the supernatant containing the RNA**!

**** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.

***** Elution with lower volumes of water (e.g. 20-30 µl) increases the final concentration of RNA. Higher volumes (80-100 µl) result in lower concentration of RNA, although the overall amount of RNA eluted may be lightly higher. Minimum elution amount is 20 µl.

5.3 RNA isolation from Yeast cells (max. 5x10^7 cells)

Count your cell number and select the appropriate cell amount, 5x10^7 cells in maximum.
Please note that depended on the strain used, parameters used for lysis in particular have to be optimised. The protocol given here represents some kind of general protocol and may have to be adapted.

Step (RT = room temperature)	**done**

1. Lysis of yeast cells	
Pellet yeast cells by centrifugation at 2.000 g (or 3.000 rpm) for approx. 5 mins. and discard the supernatant.	
Resuspend the pellet in 600 µl sorbitol buffer (see above).	
Again, pellet cells by centrifugation at 2.000 g (or 3.000 rpm) for approx. 5 mins. and discard the supernatant.*	
Resuspend the pellet in 600 µl sorbitol buffer.	
Add the appropriate amount** of (preferably) zymolyase or lyticase. Mix by pipetting up and down. Incubate at 30 °C for 60 mins. under agitation***.	
Pellet yeast cells by centrifugation at 2.000 g (or 3.000 rpm) for approx. 5 mins. and discard the supernatant.	
Resuspend the pellet in 600 µl sorbitol buffer (see above).	
Pellet yeast cells by centrifugation at 2.000 g (or 3.000 rpm) for approx. 5 mins. and discard the supernatant.	
Resuspend the pellet in 600 µl **Lysis Buffer LSR** ****, and incubate the sample for 30 mins. at RT under continuous shaking.***	

2. Removal of DNA / First Column Loading	
Precipitate unlysed material by centrifugation at max. speed for 1 min/RT.	
Place a **blue** Spin Column into a 2 ml collection tube.	
Apply the supernatant to the blue spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT. **Keep the filtrate containing the RNA** and discard the spin filter.*****	

3. Binding of RNA / Second Column Loading	
Place a **violet** Spin Column into a new 2 ml collection tube.	
Add an equal volume (approx. 600 µl) of 70 % ethanol to the filtrate from step 2. Mix by pipetting up and down.	
Apply 650 µl of the mix of filtrate/ethanol to the violet spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through.	
Apply the residual mix of filtrate/ethanol to the **same** violet spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through.******	

Sequel: RNA isolation from yeast cells

Step (RT = room temperature)	**done**

4. Column Washing	
Place the violet Spin Column into a new collection tube.	
Add 500 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through******	
Add 700 µl of **Washing Solution WSL** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through******	
Again centrifuge columns at 10.000 g (or 12.000 rpm) for 3 mins./RT in order to remove residual ethanol.	

5. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50 µl **RNase-free water** (incl. in the kit) to the centre of the membrane*******	
Incubate for 1 min at room temperature	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute RNA	

* The supernatant has to be removed as completely as possible. Remove residual medium with wipes or other appropriate measures.
** The amount required depends on the amount of cells as well as on the strain (susceptible or unsusceptible).
*** The appropriate time span for lysis has to be optimized.
**** The lysed yeast cells may be stored at -80 °C. Short term storage may be done at -20 °C.
***** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time. The Spin Filter contains the DNA and is discarded. **Do NOT discard the supernatant containing the RNA**!
****** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.
******* Elution with lower volumes of water (e.g. 20-30 µl) increases the final concentration of RNA. Higher volumes (80-100 µl) result in lower concentration of RNA, although the overall amount of RNA eluted may be lightly higher. Minimum elution amount is 20 µl.

5.4 RNA isolation from prokaryotic (bacterial) cells (max. $1x10^9$ cells)

We recommend to preincubate bacteria in lysozyme or other lysis enzymes in order to optimise the RNA recovery rate.

Step (RT = room temperature)	**done**

1. Lysis of bacterial cells	
Pellet bacterial cells by centrifugation at 5.000 g (or 7.500 rpm) for approx. 3 mins and discard the supernatant.*	
Resuspend the pellet in 100 µl 1x TE buffer (see above).	
a) For gram- bacteria: Add 2 µl **lysozyme solution (20 mg/ml**). Mix by pipetting up and down a few times until the solution becomes clear or viscous.**	
b) For gram+ bacteria: Add 6 µl **lysozyme solution (50 mg/ml).** Mix by pipetting up and down. Incubate at RT until the solution becomes clear or viscous.**	
Add 450 µl **Lysis Buffer LSR** and mix vigorously by vortexing or heavy pipetting.	
Incubate further (approx. 3 mins) at RT until the sample is *completely* lysed.***	

2. Removal of DNA / First Column Loading	
Place a **blue** Spin Column into a 2 ml collection tube.	
Apply the lysed cell solution to the blue spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 2 min/RT. **Keep the filtrate containing the RNA** and discard the spin filter.****	

3. Binding of RNA / Second Column Loading	
Place a **violet** Spin Column into a new 2 ml collection tube.	
Add an equal volume (approx. 600 µl) of 70 % ethanol to the filtrate from step 2. Mix by pipetting up and down.	
Apply 650 µl of the mix of filtrate/ethanol to the violet spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through.	
Apply the residual mix of filtrate/ethanol to the **same** violet spin column.	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through.*****	

4. Column Washing	
Place the violet Spin Column into a new collection tube.	
Add 500 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through*****	
Add 700 µl of **Washing Solution WSL** to the Spin Column	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through*****	
Again centrifuge columns at 10.000 g (or 12.000 rpm) for 3 mins/RT in order to remove residual ethanol.	

Sequel: RNA isolation from bacteria

Step (RT = room temperature)	**done**

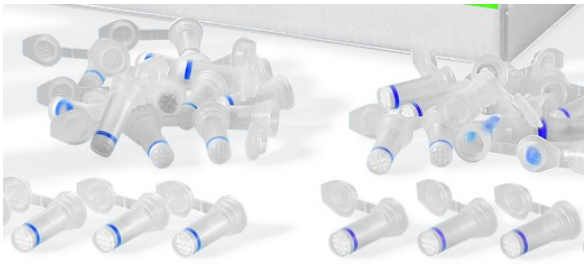
5. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50 µl **RNase-free water** (incl. in the kit) to the centre of the membrane******	
Incubate for 1 min at room temperature	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute RNA	

* The supernatant has to be removed as completely as possible. Remove residual medium with wipes or other appropriate measures.
** Incubation should not be necessary for gram cells. In case the solution doesn't become clear or viscous, incubate for a few minutes at RT. The appropriate time needed for cell lysis depends on the bacterial strain and has to be optimised. A complete destruction of the bacterial cell wall is important in order to achieve good recovery rates, so don't be impatient.
*** The appropriate time span for further lysis has to be optimized. Check lysis efficiency before proceeding in the protocol. If the lysis is not complete, prolong the incubation time in Lysis Buffer LSR. **No cell clumps should be visible after this step!** This step is critical, don't be impatient. The lysed cells may be stored in Lysis Buffer LSR at -80 °C. Short term storage may be done at -20 °C.
**** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time. The Spin Filter contains the DNA and is discarded. **Do NOT discard the supernatant containing the RNA**!
***** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.
****** Elution with lower volumes of water (e.g. 20-30 µl) increases the final concentration of RNA. Higher volumes (80-100 µl) result in lower concentration of RNA, although the overall amount of RNA eluted may be lightly higher. Minimum elution amount is 20 µl.

5. Trouble Shooting

Problem / probable cause	Comments and suggestions
Clogged spin filter	
Insufficient lysis/homogenisation and/or too much starting material	Increase lysis time.
	Increase centrifugation speed.
	After lysis centrifuge the lysate to pellet unlysed material. Continue with the supernatant.
	Use the recommended techniques for lysis of cell pellet.
	Reduce amount of starting material. Overload of filters reduces yield.
Low recovery	
Insufficient lysis/homogenisation	See above
Incomplete elution	Prolong incubation time with RNase-free water to 5 minutes, or repeat elution step once again.
Degraded RNA	
RNA source inappropriately handled or stored	See above.
	Check condition of the starting material. Is it fresh and undamaged?
	RNA is very temperature sensitive. Make sure the protocol, especially the first steps, are performed as long as necessary, but as short as possible.
RNase contamination of solutions, Collection Tubes, etc.	Use sterile, RNase-free filter tips. Before every preparation clean up the pipette, the devices and the working place. Always wear gloves!
Old material	Use fresh material. Check and improve storage conditions.
Incorrect storage of starting material.	Make sure that the starting material is frozen immediately in liquid N_2 or in minimum at -20 °C, and is stored continuously at -80 °C!
	Avoid repeated freezing and thawing of the starting material.
DNA contamination	
Insufficient lysis/homogenisation and/or too much starting material	See above.
	Perform DNase digestion of the eluate containing the total RNA. Alternatively, perform an on-column DNase digest step after binding of the RNA on the violet Spin Column.
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 Buffer WSA and Washing Solution WSL are at room temperature.
	Check Washing Solutions for salt precipitates. Dissolve these by careful warming.

Ordering information:
(for detailed kit content see Table under 1.)



ROTI®Prep RNA MINI	**10 preps (Mini kit)**	**8485.1**
ROTI®Prep RNA MINI	**50 preps (Kit)**	**8485.2**
ROTI®Prep RNA MINI	**250 preps (Maxi kit)**	**8485.3**

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