Instructions for use

ROTI®Prep Plant RNA

20H7

Kit for RNA isolation from various plant tissues

1. Introduction and product description

- Developed specifically for extraction from plant materials
- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Preparation in approx. 35 min
- Yield up to 70 µg RNA, depending on source material
- High purity: approx.1.8-2.1

ROTI®Prep Plant RNA was designed to isolate total RNA from various plant starting materials.

The isolation is performed from homogenised plant material, which is then treated with one of the lysis buffers included in the kit to break down even complex plant components. The lysed sample is then filtered through a centrifugation column, which binds the DNA. The filtrate is then transferred to a second centrifugation column that binds the RNA. After several washing and centrifugation steps, the RNA is eluted from the membrane by the elution buffer and is now ready for use in all subsequent applications.

Suitable source material:

- Blossoms
- Leaves
- Roots
- Stems
- Wooden parts
- Seeds
- Grains

→ 100 mg fresh, frozen, dried plant material can be used to extract the RNA. If the plant material is very wet, up to 180 mg are recommended

2. Product use and warranty

2.1 Intended Use

- For research use only.
- Please read the instructions for use carefully and follow the instructions accordingly. Reliability of results cannot be guaranteed if there are any deviations in the procedure.

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

2.2 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.

Caution:

Lysis Buffer LSR  Warning H302-H312-H314-H318-H332

Lysis Buffer LSK  Warning H302-H315-H319-H332

Washing Buffer WSA  Warning H302-H312-H314-H318-H332

Attention: Do not add bleach or acidic components to the waste after sample preparation!

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

3. Materials provided in this kit and storage conditions

3.1. Included Kit components

Amount	Component	Storage
6 / 30 / 125 ml	Lysis Buffer LSR	RT
6 / 30 / 125 ml	Lysis Buffer LSK	RT
3 / 15 / 70 ml	Washing Buffer WSA (conc.)	RT
3 / 15 / 2x 40 ml	Washing Solution WSL (conc.)	RT
2 / 6 / 2x 15 ml	RNase-free Water	RT
10 / 50 / 5x 50	Mini spin columns DNA (blue)	RT
10 / 50 / 5x 50	Mini spin columns RNA (violet)	RT
10 / 50 / 5x 50	1.5 ml Elution tubes	RT
60 / 6x 50 / 30x 50	2 ml Collection tubes	RT

The ROTI®Prep Plant RNA 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 user is responsible to validate the performance of the ROTI®Prep Plant RNA Kit for any particular use, since the performance characteristics of our kits have not been validated for any specific application.

3.2. 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)

4. Application

ROTI®Prep Plant RNA Kit is designed for isolation of high-purity RNA from various sources of plant material. 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 70 µg** RNA per preparation from a large variety of different plant tissues. The Kit was tested for isolation of gDNA from blossoms, fruits, leaves, needles, woods, as well as seeds. If plant tissue will not be used immediately after harvesting, it can be stored in liquid nitrogen, lyophilized/dried or frozen. Fresh material can be kept at 4 °C for 24 hours but should be frozen at -22 °C to -18 °C for longer storage. Ground tissue powder can be stored at -80 °C. Alternatively, tissue can be dried or lyophilized after harvesting to allow storage at room temperature (15 °C to 30 °C). To ensure DNA quality, samples should be completely dried within 24 hours of collection.

During lysis, the sample has to be mixed carefully and, if possible, constantly. Reduced movement of the lysis mix will reduce the lysis efficiency, and, subsequently, the recovery rate of RNA. We recommend using a shaking platform or thermomixer in order to keep the sample at constant movement.

Store the extracted RNA at -20 °C. For long time storage placing at -80 °C is recommended.

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.

4.1 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.

4.2. Before start, be sure to…

- **Add Ethanol** (96-99.8 %, not incl. in this kit) to the Washing Buffer WSA (conc.) as follows:
  20H7.1 (10 Preps): 3 ml (6 ml final vol.)
  20H7.2 (50 Preps): 15 ml (30 ml final vol.)
  20H7.3 (250 Preps): 70 ml to each bottle (2x 140ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!
- **Add Ethanol** (96-99.8 %, not incl. in this kit) to the Washing Buffer WSL (conc.) as follows:
  20H7.1 (10 Preps): 12 ml (15 ml final vol.)
  20H7.2 (50 Preps): 60 ml (75 ml final vol.)
  20H7.3 (250 Preps): 160 ml to each bottle (2x 200 ml final vol.)
  Mix thoroughly!
- For Lysis steps we recommend using a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3–4 times during the incubation. No shaking will reduce the lysis efficiency.

4.3. Homogenization and lysis of plant samples

We recommend to collect young plant materials (e.g., leaves, needles) since they contain more cells per weight and therefore extraction result in higher yields. In addition, young leaves and needles contain smaller amounts of polysaccharides and polyphenolics and are therefore easier to handle.

Complete and quick disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation. The lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include any type of commercial homogenizers (rotor-stator homogenizer) or bead mills (e.g. Cell disruptors Genie®, PA66.1).

4.3.1 Disruption of plant material

4.3.1.1 Disruption by using bead mill homogenizers

Use ROTI®SampleLyse Soft Tissue 2 (1YK5) for soft plant material and leaves or ROTI®SampleLyse Plant (1YKA) for needles or seeds. Pipette 50 µl ddH_2O to the plant material and vortex for about 30 seconds (e.g. Cell disruptors Genie®, PA66.1). Repeat the chilling and vortexing procedure until the entire plant material is ground to a fine solution.

It is also possible to chill the tube in liquid nitrogen. After the homogenization, describe above, chill the tube once more and remove the beads by rolling them out gently or using a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube since this leads to sticking and loss of plant material attached to the beads.

4.3.1.2 Disruption by using a mortar and pestle

Use mortar and pestle to grind the plant material in the presence of liquid nitrogen. Freeze plant material in liquid nitrogen and be careful during homogenization, because do not let the sample thaw at any time. We recommend precooling the used laboratory equipment. Grind frozen plant sample to a fine powder and refill mortar with liquid nitrogen to keep the sample frozen, if necessary. Use precooled tubes for sample storage until lysis step, but make sure no liquid nitrogen is transferred or all nitrogen has evaporated before closing the tube. Proceed directly with the lysis step and don't allow the sample to thaw.

4.3.1.2 Disruption by using bead mill homogenizers

Use ROTI®SampleLyse Soft Tissue 2 (1YK5) for plant material and leaves or ROTI®SampleLyse Plant (1YKA) for needles or seeds. Pipette 450 µl Lysis Buffer to the plant material and vortex for about 30 seconds (e.g. Cell disruptors Genie®, PA66.1). Repeat the chilling and vortexing procedure until the entire plant material is ground to a fine solution.

It is also possible to chill the tube in liquid nitrogen. After the homogenization, describe above, chill the tube once more and remove the beads by rolling them out gently or using a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube since this leads to sticking and loss of plant material attached to the beads.

4.3.2 Lysis of plant samples

Plants are very heterogeneous and contain varying amounts of polyphenols, acidic components, or polysaccharides which can lead to suboptimal DNA extraction or performance in downstream applications. Therefore, two different lysis buffers are provided for optimal processing, purification performance, high yields and an excellent DNA quality for the most common plant species.

The standard protocol uses Lysis Buffer LSR. However, for for some plant species Lysis Buffer LSK works better. Please start the extraction process with Lysis Solution LSR. In case of low yield or no yield please use the second Lysis Solution LSK.

4.4 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.

4.3.1 RNA isolation from plant material

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

1. Lysis of plant material	
Homogenize plant material using one of the methods described in section 4.2.1, p.3	
Transfer homogenized plant material in a 1.5 ml reaction tube	
If you have not done so: Add 400 µl **Lysis Buffer LSR** or **Lysis Buffer LSK** and mix vigorously by pulsed vortexing for 5 s.	
Transfer sample on a pre-filtration column located in a collection tube.	
Centrifuge at full-speed for 1 min to pellet down unlysed material.	

2. Column Loading	
Place each Spin Column DNA (blue ring) into a 2 ml collection tube	
Apply the supernatant to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT. Discard the spin column and keep the flow-through as it contains the RNA.	
Apply an equal volume of 70% ethanol to the flow-through and mix by pipetting up and down	
Place each Spin Column RNA (violet ring) into a fresh 2 ml collection tube	
Transfer the flow-through/ethanol mix to the spin column	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through and the collection tub	

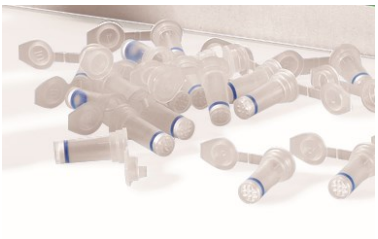
3. Column Washing	
Place the Spin Column RNA into a new 2 ml collection tube	
Add 500 µl of **Washing Buffer WSA** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube.	
Place the Spin Column RNA into a new 2 ml collection tube	
Add 650 µl of **Washing Buffer WSL** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube.	
Place the Spin Column RNA into a new 2 ml collection tube	
Add 650 µl of **Washing Buffer WSL** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube.	
Place the Spin Column into a new 2 ml collection tube	
Againcentrifuge columns at 12.000-14.000 rpm (or full speed) for 2 mins/RT in order to remove residual ethanol. Discard the collection tube	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 30-80 µl **RNase-free Water** to the centre of the membrane.	
Incubate for 1 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA	

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 Plant RNA	**10 preps**	**20H7.1**
ROTI®Prep Plant RNA	**50 preps**	**20H7.2**
ROTI®Prep Plant RNA	**250 preps**	**20H7.3**

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LHa 03/2023