Instructions for use

ROTI®Prep DNA & RNA

20H8

Kit for simultaneous isolation of DNA and RNA from different starting materials.

1. Introduction and product description

- Simultaneous isolation of DNA and RNA from one sample
- Preparation in the well-established mini-column system
- Fast, easy and reliable
- Extraction time of approx. 8 min (excluding lysis)
- Average puritiy DNA: 1.7-2.0, RNA: 1.8-2.1

ROTI®Prep DNA & RNA was developed to isolate both DNA and RNA separately from only one sample.

The isolation is based on the use of two different centrifugation columns. After sample lysis, the sample is first transferred to the first centrifugation column, which binds the DNA. The lysate is then transferred to a second centrifugation column, to whose membrane the RNA binds. Subsequently, DNA and RNA can be washed in parallel in the centrifugation columns and then eluted in separate reaction vessels.

Suitable source material:

- Microbial cell cultures (max. $5x10^9$)
- Eucaryotic cell cultures (max. 5^{10})
- Tissue samples (max. 20 mg)

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 DNA & 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-H304-H3012-H3014-H3018-H332

Washing Buffer WSA (conc)  Warning H302-H304-H3012-H3014-H3018-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 / 2x 70 ml	Washing Buffer WSA (conc.)	RT
3 / 2x 8 / 2x 40 ml	Washing Buffer WSL (conc.)	RT
2 / 6 / 25 ml	RNase free Water	RT
2 / 6 / 30 ml	Elution Buffer EB	RT
10 / 50 / 5x 50	Mini spin columns DNA (blue)	RT
10 / 50 / 5x 50	Mini spin columns RNA (violet)	RT
20 / 2x 50 / 10x 50	1.5 ml Elution tubes	RT
40 / 4x 50 / 20x 50	2 ml Collection tubes	RT

The ROTI®Prep DNA & 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 DNA & 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)
- TE buffer (e.g.1052.1)
- Lysozym (400 U/µl) for enzymatic lysis of bacteria (e.g. 8259.1)

4. Application

ROTI®Prep DNA & RNA Kit is designed for isolation of high-purity genomic DNA from various source 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 **40 µg** DNA and **60 µg** RNA per preparation from microbial cell cultures (max. $1x10^9$), fungi and eukaryotic cell cultures (max. $5x10^6$), up to 20 mg Tissue samples.

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 gDNA. We recommend using a shaking platform or thermomixer in order to keep the sample at constant movement.

Store the extracted DNA at +4 °C and the extracted RNA at -20°C. For long time storage placing at -20 °C (DNA) or -80 °C (RNA) 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:
  20H8.1 (10 Preps): 6 ml (12 ml final vol.)
  20H8.2 (50 Preps): 30 ml (60 ml final vol.)
  20H8.3 (250 Preps): 70 ml to each bottle (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 (conc.) as follows:
  20H8.1 (10 Preps): 12 ml (15 ml final vol.)
  20H8.2 (50 Preps): 32 ml to each bottle (2x 40 ml final vol.)
  20H8.3 (250 Preps): 160 ml to each bottle (2x 200 ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!

- Heat one thermal mixer or water bath to 56 or 60 °C.
- Pre-fill the needed amount of RNase free water or Elution Buffer into a 2.0 ml reaction tube and incubate at 60 °C until the elution step.
- 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. 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 DNA isolation from bacterial cell cultures

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

1. Collection of bacterial cells	
Transfer the bacterial cell culture into a 2.0 ml reaction tube	
Pellet cells by centrifugation at 5000 g (or 7000 rpm) for 5 mins and discard the supernatant	
Resuspend the bacterial cell pellet in 100 µl TE-Buffer.	

2.1 Lysis of bacterial cells	
Add 20 µl **Lysozyme** (10mg/ml, 400 U/µl) and incubate at 37 °C for 30 minutes under continuous shaking (obligatory step for gram-positive bacteria, optional step for gram-negative bacteria). For Staphylocuccus 10 µl **Lysostaphin** (0.4 U/µl) are recommended	
Add 450 µl **Lysis Buffer LSR** to the sample and vortex the sample shortly	
Incubate the sample for 3 minutes at RT	
Centrifuge at max. speed for 1 min to spin down the unlysed material. Do not discard the supernatant	

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. *Note:* Do not discard the flowthrough as it contains the RNA. Also keep the spin column as is contains the DNA	
Add an equal volume of 70 % ethanol (p.a.) to the flow-through and mix by carefully pipetting up and down.	
Place each **Spin Column RNA (violet ring)** into a fresh 2 ml collection tube	
Transfer the flowthrough/ethanol mix (max. 650 µl) to the spin column RNA	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flowthrough and place the spin column back into the collection tube spin. Repeat the last centrifugation step if there is any residual sample.	

3. Column Washing (DNA and RNA in parallel)	
Place the **Spin Column DNA (blue ring)** into a new 2 ml collection tube. It is washed in parallel with the S**pin column RNA (violet).**	
Add 500 µl of **Washing Buffer WSA** to the Spin Column DNA and to Spin Column RNA	
Centrifuge both vessels at 11.000 g (or 13.000 rpm) for 1 min/RT, discard the flow-through and reuse the collection tube	
Place the Spin Column back into the 2 ml collection tube	
Add 700 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 6000 g (or 8000 rpm) for 1 min/RT, discard the flow-through and reuse the collection tube	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 2mins/RT in order to remove residual ethanol.	

4. Elution	
Place each Spin Column into a clean 1.5 ml elution tube	
Add 30-80 µl **RNase free Water** to spin column RNA (violet) and 100 µl **Elution Buffer** to spin column DNA (blue ring) the centre of the membrane.	
Incubate for 2 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	

4.3.2 DNA isolation from eucaryotic cell cultures

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

1. Collection of cells	
Transfer up to 5 x 10^6 eukaryotic cells into a 2.0 ml reaction tube. Pellet cells by centrifugation at 5000 g (or 7000 rpm) for 5 mins and discard the supernatant.	

2.1 Lysis of cells cells	
Add 400 µl **Lysis Buffer LSR** to the sample and vortex the sample shortly	
Incubate the sample for 2 min at RT. Resuspend the cells completely by pipetting up and down. Incubate the sample for further 3 min at RT. *Note:* In order to obtain maximum yield of DNA and RNA the cells should be completely lysed to proceed	
Centrifuge at max. speed for 1 min to spin down the unlysed material. Do not discard the supernatant	

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. *Note:* Do not discard the flowthrough as it contains the RNA. Also keep the spin column as is contains the DNA	
Add an equal volume of 70 % ethanol (p.a.) to the flow-through and mix by carefully pipetting up and down.	
Place each **Spin Column RNA (violet ring)** into a fresh 2 ml collection tube	
Transfer the flowthrough/ethanol mix (max. 650 µl) to the spin column RNA	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flowthrough and place the spin column back into the collection tube spin. Repeat the last centrifugation step if there is any residual sample.	

3. Column Washing	
Place the **Spin Column DNA (blue ring)** into a new 2 ml collection tube. It is washed in parallel with the S**pin column RNA (violet).**	
Add 500 µl of **Washing Buffer WSA** to the Spin Column DNA and to Spin Column RNA	
Centrifuge both vessels at 11.000 g (or 13.000 rpm) for 1 min/RT, discard the flow-through and reuse the collection tube	
Place the Spin Column back into the 2 ml collection tube	
Add 700 µl of **Washing Buffer WSL** to the Spin Column	
Centrifuge at 6000 g (or 8000 rpm) for 1 min/RT, discard the flow-through and reuse the collection tube	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 2mins/RT in order to remove residual ethanol.	

4. Elution	
Place each Spin Column into a clean 1.5 ml elution tube	
Add 30-80 µl **RNase free Water** to spin column RNA (violet) and 100 µl **Elution Buffer** to spin column DNA (blue ring) the centre of the membrane.	
Incubate for 2 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	

4.3.3 DNA isolation from tissue samples

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

2.1 Lysis of cells cells	
Cut max. 20 mg of tissue/biopsie sample into small pieces and homogenize the sample. Homogenization can either be performed by: • Using mortar pestle (grind with liquid nitrogen and avoid thawing, then transfer the sample to a reaction tube and add 450 µl **Lysis Buffer LSR** • Using a SampleLyse Tube e.g. 1YKA.1 and a speed mill. If you are doing this you can add the **Lysis Buffer LSR** (450 µl) directly to the sample in the lysis tube. Homogenize the sample in a rotor stator mill. *Note:* In order to obtain maximum yield of DNA and RNA the cells should be completely lysed to proceed	
Centrifuge at max. speed for 1 min to spin down the unlysed material. Do not discard the supernatant	

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. *Note:* Do not discard the flowthrough as it contains the RNA. Also keep the spin column as is contains the DNA	
Add an equal volume of 70 % ethanol (p.a.) to the flow-through and mix by carefully pipetting up and down.	
Place each **Spin Column RNA (violet ring)** into a fresh 2 ml collection tube	
Transfer the flowthrough/ethanol mix (max. 650 µl) to the spin column RNA	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flowthrough and place the spin column back into the collection tube spin. Repeat the last centrifugation step if there is any residual sample.	

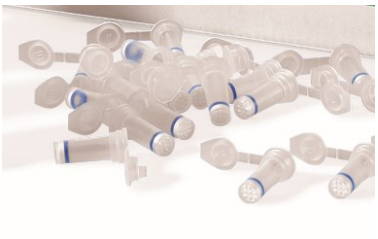
3. Column Washing	
Place the **Spin Column DNA (blue ring)** into a new 2 ml collection tube. It is washed in parallel with the S**pin column RNA (violet).**	
Add 500 µl of **Washing Buffer WSA** to the Spin Column DNA and to Spin Column RNA	
Centrifuge both vessels at 11.000 g (or 13.000 rpm) for 1 min/RT, discard the flow-through and reuse the collection tube	
Place the Spin Column back into the 2 ml collection tube	
Add 700 µl of **Washing Buffer WSL** to the Spin Column	
Centrifuge at 6000 g (or 8000 rpm) for 1 min/RT, discard the flow-through and reuse the collection tube	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 2mins/RT in order to remove residual ethanol.	

4. Elution	
Place each Spin Column into a clean 1.5 ml elution tube	
Add 30-80 µl **RNase free Water** to spin column RNA (violet) and 100 µl **Elution Buffer** to spin column DNA (blue ring) the centre of the membrane.	
Incubate for 2 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
1. Clogged spin filter	
Insufficient lysis and/or too much starting material	Increase lysis time.
	Increase centrifugation speed.
	After lysis centrifuge the lysate to pellet unlysed material.
	Check storage conditions and usage of Proteinase K. Optionally replace Proteinase K by a fresh lot.
	Reduce amount of starting material. Overload of filters reduces yield.
2. Low recovery	
Insufficient lysis	See above
Insufficient mixing with Binding Buffer BSN	Mix sample very well with Binding Buffer BSN by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter
Low amount of recovered gDNA.	Add the Elution Buffer directly onto the centre of the Spin Column.
	Prolong the incubation time with Elution Buffer.
	Increase volume of Elution Buffer used or repeat elution step.
Low concentration of eluted gDNA	Reduce the volume of Elution Buffer used. Optionally: prolong elution / incubation time.
3. Degraded or sheared DNA	
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.
Old material	Use fresh material. Check and improve storage conditions.
4. Poor quality of RNA	
Ethanol carry-over during elution	Increase time for removing ethanol
	Ensure that both Washing buffers have room temperature. Check up Washing Buffers for salt precipitates. If there are any precipitate dissolve these precipitate by carefully warming.

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



ROTI®Prep DNA & RNA	**10 preps**	**20H8.1**
ROTI®Prep DNA & RNA	**50 preps**	**20H8.2**
ROTI®Prep DNA & RNA	**250 preps**	**20H8.3**

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LHa 03/2023