Instructions for use

ROTI®Prep Soil DNA

20H9

Kit for isolation of microbial DNA from soil samples

1. Introduction and product description

- Specially developed for DNA extraction from complex soil samples
- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Preparation in approx. 45 min
- Average Ratio A_{260}:A_{280}: 1.7-2.0

ROTI®Prep Soil DNA was developed to isolate microbial DNA from soil samples. Soil samples represent a particularly complex starting material, which is why it is urgently necessary to first mechanically decompose it. In the first step of extraction, the soil samples are homogenized and disrupted in a lysis tube with lysis buffer. After a centrifugation step, the lysate is transferred to the centrifugation column and the DNA is bound to the membrane with the aid of the binding buffer. After several washing and centrifugation steps, the DNA is then eluted from the membrane with the Elution Buffer EB. The extracted DNA can be used for different downstream applications like PCR, qPCR, Digital PCR or Nanopore Sequencing.

Suitable source material:

Up to 400 mg of soil sample.

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 Soil DNA 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 LSS  Warning H319-H302-H315-H412-H319

Precipitation Buffer PB  Danger H226-H314-H315-H319

Binding Buffer BR  Danger H225-H319-H336

Binding Buffer BDS  Danger H314-H302-H312-H332-H412

Washing Solution WSO  Danger H225-H319-H336

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
10 / 50	Roti®SampleLyse Microbes tube	RT
9 / 45 ml	Lysis Buffer LSS	RT
2 / 6 ml	Precipitation Buffer PB	RT
10 / 50 ml	Binding Buffer BR	RT
3 / 11 ml	Binding Buffer BDS	RT
8 / 50 ml	Washing Solution WSO	RT
1 / 5 ml	Washing Buffer WSH (conc.)	RT
2x 2 / 12 ml	Elution Buffer EB	RT
20 / 2x 50	Mini spin columns	RT
20 / 2x 50	1.5 ml Elution tubes	RT
20 / 2x 50	2 ml Collection tubes	RT

The ROTI®Prep Soil DNA 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 Soil DNA 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)
- A homogenizer or Speed Mill (e.g. PA66.1)

4. Application

ROTI®Prep Soil DNA Kit is designed for efficient isolation of high-purity environmental DNA from different soil samples. 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.

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 DNA.

Store the extracted DNA at +4 °C. For long time storage placing at -20 °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. Before start, be sure to…

- **Add Ethanol** (96-99.8 %, not incl. in this kit) to the Washing Buffer WSH (conc.) as follows:
  20H9.1 (10 Preps): 9 ml (10 ml final vol.)
  20H9.2 (50 Preps): 45 ml (50 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 Elution Buffer EB 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.

4.2. 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.

4.2.1 DNA exctraction from soil samples

Step (RT = room temperature)	done

1. Lysis of soil material	
Transfer max. 400 mg soil starting material into a **Roti®SampleLyse Microbes 1** tube.	
Add 800 µl **Lysis Buffer LSS** and mix vigorously by pulsed vortexing for 5 s. Place the lysis tube in a homogenizer and homogenize the material for 1 min. *The homogenization process can be changed and optimized depending on the used homogenizer. The optimal duration and intensity of homogenization depends on kind of homogenizer used.*	
Place the Roti®SampleLyse Microbes 1 tube in a centrifuge and spin at max. speed for 7 min	
Transfer the supernatant carefully into a new 2.0 ml reaction tube. Avoid carry-over of pellet-material.	
Add an equal volume of **Binding Buffer BR** to the sample. Mix by pipetting up and down until a *homogenous* solution is achieved.	

2. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply 750 µl of the mix of sample/ Binding Buffer BR to the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and repeat the step with the rest of the sample. Reuse the collection tube.	

3. Column Washing	
Add 700 µl of **Washing Solution WSO** to the Spin Column and incubate for 1 min/RT	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through.	
Add 700 µl of **Washing Buffer BS** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through	
Place the Spin Column back into the collection tube	
Add 400 µl of absolute ethanol	
Centrifuge at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol, discard the flow-through and the collection tube.	

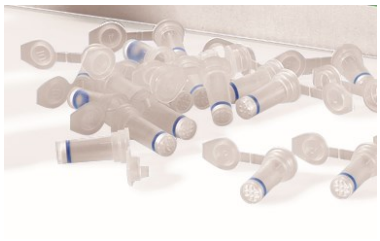
4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50-100 µl preheated (70 °C) **Elution Buffer EB** to the centre of the membrane.	
Incubate for 2 min at room temperature	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT to elute DNA	
Repeat the elution step (by loading the eluted DNA again on the spin column) to increase the yield of extracted DNA. *If the eluat is discoloured, use the following cleanup protocol (p.4)*	

5. Clean up protocol	
Add 200 µl Binding Buffer RBS to the eluate, mix by pipetting up and down until a *homogenous* solution is achieved.	
Apply the sample to in a spin column located in a collection tube and centrifuge at 11,000 x g for 1 minute. Discard the flow-though and place the spin column back in the collection tube.	
Add 750 µl 80% ethanol and centrifuge at 11.000 x g for 1 min. Discard the flow-though and place the spin column back in the collection tube.	
Again add 750 µl 80% ethanol and centrifuge at 11.000 x g for 1 min. Discard the flow-though and place the spin column back in the collection tube.	
Centrifuge at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol, discard the flow-through and the collection tube.	
Add 50-100 µl preheated (70 °C) **Elution Buffer EB** to 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	

5. Trouble Shooting

Problem / probable cause	Comments and suggestions
1. Clogged spin filter	
Insufficient lysis and/or too much starting material	After lysis centrifuge the lysate to pellet unlysed material.
	Reduce amount of starting material. Overload of filters reduces yield.
2. Low recovery	
Insufficient homogenization	Increase homogenization time and/or speed.
Insufficient lysis	See above
Insufficient mixing with Binding Buffer BL	Mix sample very well with Binding Buffer BR by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter
Incomplete elution	Add the Elution Buffer EB directly onto the centre of the Spin Column.
	Prolong the incubation time with Elution Buffer EB.
	Increase volume of Elution Buffer EB used or repeat elution step.
Low concentration of eluted gDNA	Reduce the volume of Elution Buffer EB used. Optionally: prolong elution / incubation time.
4. Elutes are discoloured	
Incorrect washing steps	Perform Clean up protocol

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



ROTI®Prep Soil DNA	**10 preps**	**20H9.1**
ROTI®Prep Soil DNA	**50 preps**	**20H9.2**

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