Instructions for use

ROTI®Prep Plant gDNA

20H4

Kit for isolation of genomic DNA 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 25 µg gDNA, depending on source material
- Average Ratio A_{260}:A_{280}: 1.7-2.0

ROTI®Prep Plant gDNA has been designed to isolate DNA from various sources of plant material.

ROTI®Prep Plant gDNA was developed to isolate genomic DNA from various plant sources. The isolation is performed from homogenised plant material, which is then treated with one of the lysis buffers included in the kit. The kit contains three different lysis buffers, each containing a mixture of chaotropic and anti-chaotropic salts, in order to optimally cope with the diversity of plant starting materials. After lysis, a pre-filtration step is performed to filter out the remaining cell debris and contaminants from the lysate. The lysate is then mixed with the binding buffer, which allows the DNA to bind efficiently to the membrane of the centrifugation columns. After several washing and centrifugation steps, the DNA 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

→ 120-180 mg fresh plant material or 50-100 mg dried plant material can be used to extract the DNA.

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 gDNA 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 LSN Warning H319-H400-H412

Lysis Buffer LSP Warning H319

Lysis Buffer LSV Danger H318

Precipitation Buffer PB Warning H315-H319

Binding Buffer BSN Danger H225-H319-H336

Proteinase K Danger H315-H319-H334-H317-H335

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
5 / 25 / 120 ml	Lysis Buffer LSN	RT
5 / 25 / 120 ml	Lysis Buffer LSP	RT
5 / 25 / 120 ml	Lysis Buffer LSV	RT
2 / 8 / 30 ml	Precipitation Buffer PB	RT
5 / 15 / 60 ml	Binding Buffer BSN	RT
For 1x 0.3 / 1x 1.5 / 4x 1.5 ml	Proteinase K	+4 °C
6 / 24 / 2x 60 ml	Washing Buffer WST (conc.)	RT
4 / 15 / 60 ml	Elution Buffer EB	RT
10 / 50 / 5x 50	Pre-filtration columns	RT
10 / 50 / 5x 50	Mini spin columns	RT
10 / 50 / 5x 50	1.5 ml Elution tubes	RT
50 / 5x 50 / 25x 50	2 ml Collection tubes	RT

- Lyophilized **Proteinase K** should be stored at +4 °C. Prior to use, dissolve Proteinase K in 1x 0.3 / 1x 1.5 / 4x 1.5 ml sterile, distilled water as given below. Dissolved Proteinase K should be stored in aliquots at 4-8 °C. Avoid repeated freeze&thaw cycles for each tube.

The ROTI®Prep Plant gDNA 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 gDNA 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)
- Sterile, nuclease-free ddH_2O (e.g. 1HPE.1)
- RNase A (100 mg/ml, e.g. 7156.3)

4. Application

ROTI®Prep Plant gDNA Kit is designed for isolation of high-purity genomic DNA 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 25 µg** gDNA 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 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. 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 Solution MS (conc.) as follows:
  20H4.1 (10 Preps): 14 ml (20 ml final vol.)
  20H4.2 (50 Preps): 56 ml (80 ml final vol.)
  20H4.3 (250 Preps): 140 ml to each bottle (2x 200 ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!
- **Dissolve Proteinase K** by addition of distilled H_2O as follows:
  20H4.1 (10 Preps): 0.3 ml
  20H4.2 (50 Preps): 1.5 ml
  20H4.3 (250 Preps): 1.5 ml to each tube
  Mix thoroughly!

- 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.

4.2. 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). However, we recommend grinding with a mortar and pestle in the presence of liquid nitrogen to obtain optimal yields. When using tissues other than leaves, the disruption method may require optimization to ensure maximum DNA yield and quality. After homogenization and treatment of the sample with lysis solution, the crude lysate can be cleared easily either with Prefilters or by centrifugation.

4.2.1 Disruption of plant material

4.2.1.1 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.

4.2.1.2 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 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.

4.2.1.3 Disruption by using rotor-stator homogenizers

Rotor-stator homogenizers are only useful to disrupt soft plants in the presence of lysis solution. Keep homogenizer submerged at all times to reduce foaming.

4.2.2 Lysis of plant samples

Increasing the amount of starting material

The standard protocols of ROTI®Prep Plant gDNA Kit allow processing of 120-180 mg (wet weight) or 50-100 mg (dry weight) of plant material. This usually yields around 25 µg of high quality gDNA. However, the amount of DNA that can be expected per mg of sample depends on the size and ploidy of the genome.

To obtain sufficient DNA yield, it might be advantageous to process a higher than the recommended sample mass. However, to ensure complete lysis, all lysis solution volumes of protocol step 2 have to be increased proportionally and require multiple loading steps.

4.2.2.1 Selecting the optimal lysis system

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, three different lysis solutions 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 LSN, containing CTAB as detergent component. Additionally, the SDS based Lysis Buffer LSP is provided which requires subsequent precipitation step to remove all impurities by Precipitation Buffer PB. For some plant species Lysis Buffer LSN and LSP can be used with similar results. In these cases, please make a choice for the easiest protocol. Further the Lysis Buffer LSV has been optimized for isolation of gDNA from seeds but can also be used for other plant materials.

In order to find optimal lysis conditions when using a certain plant sample for the first time, it is recommended to do side-by-side preparations of one batch of homogeneously ground material with the three different lysis solutions and make a decision for the best one (regarding yield, quality or other relevant parameters). The following example of application illustrates the effects of different lysis solutions on yield and quality of the extracted gDNA.

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.

- Protocol 1 (using Lysis Buffer LSN) p. 5
- Protocol 2 (using Lysis Buffer LSP) p. 6
- Protocol 3 (using Lysis Buffer LSV) p. 7

4.3.1 Protocol 1 (using Lysis Buffer LSN)

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	
Add 400 µl **Lysis Buffer LSN** and 20 µl **Proteinase K** and mix vigorously by pulsed vortexing for 5 s. Incubate at 65 °C until the sample is completely lysed (appr. 30 minutes).	
Transfer sample on a pre-filtration column located in a collection tube.	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT. Keep the Collection tube with the flowthrough.	
The kit co-purifies DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 4 µl of a **RNase A stock solution** (100 mg/ml) to the sample vortex shortly and incubate for 5 minutes at room temperature.	
Add 200 µl **Binding Buffer BSN** 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 the mix of sample/Binding Buffer BSN 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 tube***	

3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of **Washing Buffer WST** 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	
Add 650 µl of **Washing Buffer WST** 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	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 100-200 µl **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	

4.3.2 Protocol 2 (using Lysis Buffer LSP)

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	
Add 400 µl **Lysis Buffer LSP** and mix vigorously by pulsed vortexing for 5 s. Incubate at 65 °C until the sample is completely lysed (appr. 30 minutes).	
Add 100 µl **Precipitation buffer PB** and vortex for 5 s. Incubate for 5 min at RT	
Centrifuge for 5 min at full-speed	
Transfer supernatant on a pre-filtration column located in a collection tube.	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT. Keep the Collection tube with the flowthrough.	
The kit co-purifies DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 4 µl of a **RNase A stock solution** (100 mg/ml) to the sample vortex shortly and incubate for 5 minutes at room temperature.	
Add 200 µl **Binding Buffer BSN** 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 the mix of sample/Binding Buffer SBS 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 tube.	

3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of **Washing Buffer WST** 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	
Add 650 µl of **Washing Buffer WST** 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	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 3 mins/RT in order to remove residual ethanol.	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 100-200 µl **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	

4.3.3 Protocol 3 (using Lysis Buffer LSV)

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	
Add 400 µl **Lysis Buffer LSV** and 20 µl **Proteinase K** and mix vigorously by pulsed vortexing for 5 s. Incubate at 65 °C until the sample is completely lysed (appr. 30 minutes).	
Add 100 µl **Precipitation buffer PB** and vortex for 5 s. Incubate for 5 min at RT	
Centrifuge for 5 min at full-speed.	
Transfer supernatant on a pre-filtration column located in a collection tube.	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT. Keep the Collection tube with the flowthrough.	
The kit co-purifies DNA and RNA, if DNA and RNA in the sample. If RNA-free genomic DNA is required, add 4 µl of a **RNase A stock solution** (100 mg/ml) to the sample vortex shortly and incubate for 5 minutes at room temperature.	
Add 200 µl **Binding Buffer BSN** 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 the mix of sample/Binding Buffer BSNto the spin column.	
Centrifuge at 11.000 g (or 13.000 rpm) for 2 min/RT and discard the flow-through and the collection tube.	

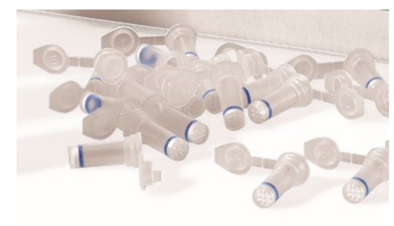
3. Column Washing	
Place the Spin Column into a new 2 ml collection tube	
Add 650 µl of **Washing Buffer WST** to the Spin Column	
Centrifuge at 11.000 g (or 13.000 rpm) for 1 min/RT and discard the flow-through and the collection tube.	
Place the Spin Column into a new 2 ml collection tube	
Add 650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

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 100-200 µl **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	

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 SBS	Mix sample very well with Binding Buffer SBS by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter
Incomplete elution	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. RNA contamination	
Unsufficient RNase digestion during lysis	Add RNase A during lysis.
	Check storage conditions and usage of RNase A. Optionally replace RNase A by a fresh lot.

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



ROTI®Prep Plant gDNA	**10 preps**	**20H4.1**
ROTI®Prep Plant gDNA	**50 preps**	**20H4.2**
ROTI®Prep Plant gDNA	**250 preps**	**20H4.3**

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