Instructions for use

ROTI®Prep Gel & PCR

20H6

Kit for DNA isolation from agarose gel pieces, or from PCR and sequencing reactions.

1. Introduction and product description

- Great flexibility through dual use
- Preparation in the well-established mini-column system
- Fast, easy and reliable
- Extraction time of approx. 3 min for PCR and Sequencing reactions, 20 min for agarose gel pieces
- Fragment length: 60 bp – 30 kbp
- Rate of recovery: 60-95 %
- Average Ratio A_{260}:A_{280}: 1.7-2.0

ROTI®Prep Gel & PCR was designed to provide the greatest flexibility in the purification of isolated DNA fragments. The kit is used for the purification of DNA from PCR reactions as well as for the purification from gel pieces and requires a minimum of washing steps.
The purification from PCR and sequencing reactions is based on a new two-step process in which the DNA is first bound to the column membrane using the Binding Buffer and then eluted in the second step.
In the purification from agarose gel pieces, the gel piece is first dissolved and the DNA is then washed in several washing and centrifugation steps before it can be eluted.

Suitable source material:

- TAE and TBE agarose gels (up to 300 mg)
- PCR reactions (up to 50 µl)
- Sequencing reactions (up to 50 µl)"

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 Gel & PCR*

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:

Gel Solubilizer  Danger H314-H302-H312-H332-H412

Binding Enhancer  Danger H226-H314-H315-H319

Binding Buffer  Danger H225-H319-H336-H315

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
8 / 40 / 180 ml	Gel Solubilizer	RT
1 / 5 / 15 ml	Binding Enhancer	RT
6 / 30 / 140 ml	Binding Buffer	RT
3 / 2x 8 / 2 x 40 ml	Washing Solution WSL (conc.)	RT
2 / 2x 2 / 15 ml	Elution Buffer EB	RT
10 / 50 / 5x 50	Mini spin columns	RT
10 / 50 / 5x 50	1.5 ml Elution tubes	RT
10 / 50 / 5x 50	2 ml Collection tubes	RT

The ROTI®Prep Gel & PCR 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 Gel & PCR 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)

4. Application

ROTI®Prep Gel & PCR was developed to provide the greatest flexibility in the purification of isolated DNA fragments. 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 high purified DNA from PCR or sequencing reactions, or, alternatively from agarose gel pieces .

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.

4.1. Before start, be sure to…

- **Add Ethanol** (96-99.8 %, not incl. in this kit) to the Washing Solution WSL (conc.) as follows:
  20H6.1 (10 Preps): 12 ml (15 ml final vol.)
  20H6.2 (50 Preps): 32 ml to each bottle (40 ml final vol.)
  20H6.3 (250 Preps): 160 ml to each bottle (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 Elution Buffer EB into a 2.0 ml reaction tube and incubate at 60°C until the elution step.

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 Extraction from agarose gel slices (TAE or TBE agarose gels)

Step (RT = room temperature)	done

1. Sample Preparation	
Excise the DNA fragment from the agarose gel with a sharp scalpel and transfer it into a 1.5 or 2 ml reaction tube (max. 300 mg weight)	
Add 650 µl **Gel Solubilizer** and incubate the slice for approx. 10 min at 50 °C until the gel is completely dissolved	
Add 50 µl **Binding Enhancer** to the sample. Mix by vortexing or pipetting up and down	

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

3. Column Washing	
Place the Spin Column back into the 2 ml collection tube	
Add 700 µl of **Washing Solution WSL** 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 2 ml collection tube	
Add 700 µl of **Washing Solution 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 back into the 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 30-50 µl (or 10-20 µl for higher concentration) **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	

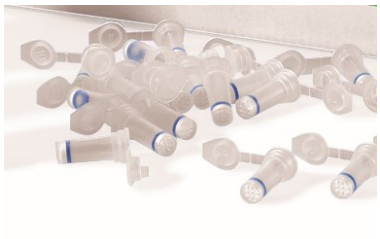
4.2.1 Purification and Concentration of PCR Products

Step (RT = room temperature)	done

1. Binding of PCR fragments	
Place each Spin Column into a 2 ml collection tube	
Add 500 µl **Binding Buffer** to the spin filter	
Add up to 50 µl PCR reaction to the spin filter. Mix by vortexing or pipetting up and down	
Centrifuge at 11.000 g (or 13.000 rpm) for 3 min/RT and discard the collection tube and the flow-through	

4. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 30-50 µl (10-20 µl for higher concentration of PCR fragments) **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	

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



ROTI®Prep Gel & PCR	**10 preps**	**20H6.1**
ROTI®Prep Gel & PCR	**50 preps**	**20H6.2**
ROTI®Prep Gel & PCR	**250 preps**	**20H6.3**

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