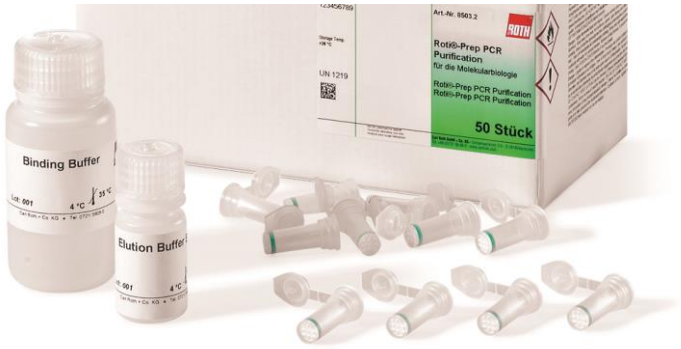
Instructions for use

ROTI®Prep PCR Purification

8503

For concentration and purification of PCR products



Introduction and product description:

- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Purification in approx. 3 minutes
- For fragment length of 60 bp-30 kb
- Recovery rate up to 95 %
- Binding capacity >20 µg
- Elution in 10 µl

Very fast purification and/or concentration of PCR products between 60 bp and 30 kb length, through simple but effective spin column technique.

The high performance membrane used for the ROTI®Prep PCR Purification columns, allows purification or concentration of PCR products within approx. 3 minutes. The PCR product to be extracted (50 µl in maximum) is first mixed with binding buffer, which can be done separately or directly in the spin filter column. The nucleic acid is then eluted by centrifugation, without any additional washing step, in a minimum of 10 µl of water or buffer.

Isolated DNA is free of residual nucleotides or PCR additives, and may directly be applied to all standard downstream applications like cloning or sequencing.

For research use only.

Caution:
Binding Buffer **Danger** H225-H319-H336
P210-P280-P303+P361+P353-P305+P338-P312

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

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.

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

1. Materials provided with the kit and storage conditions

Amount	Component	Storage
7 / 30 / 150 ml	Binding Buffer	RT
2 / 2 / 3x2 ml	Elution Buffer EB	RT
10 / 50 / 250	Mini spin columns (green)	RT
10 / 50 / 250	1.5 ml Elution tubes	RT
10 / 50 / 250	2 ml Collection tubes	RT

The ROTI®Prep PCR Purification 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 dissolve these precipitates by careful warming. This kit is stable up to 1 year after receipt, when stored as directed.

Contents of this Kit may not be bought separately.

The components of each ROTI®Prep PCR Purification Kit were tested in general purification of PCR fragments of different length. The user is responsible, however, to validate the performance of the ROTI®Prep PCR Purification Kit for any particular use, since the performance characteristics of our kits have not been validated for any specific application.

2. Required Material and Equipment not included in this kit

No additional material required

3. Application

ROTI®Prep PCR Purification Kit is designed for isolation of high-purity DNA (amplicons) from PCR Mixes for subsequent cloning, sequencing, restriction digest, or transformation. Spin column based preparation allows elution in a small volume of low-salt buffer, eliminating time-consuming other extraction protocols of even alcohol precipitation. The columns may be used either in micro-centrifuges or on vacuum manifolds.

The kit allows the recovery of up to 95 % DNA per preparation from 50 µl PCR reaction mix.

Down-scaling
For PCR mixes of 25 to 50 µl volume, the procedure may be used as described. In case smaller PCR mixes shall be used as sample, we recommend to add water in order to increase the sample volume to at least 25 µl.

Up-scaling
For up-scaling, all components should be calculated according to the factor
volume of PCR mix : 50
For centrifugation of the spin-column, the sample/Binding Buffer Mix should be split. Centrifuge approx. half of the mixture first and discard the flow-through, then centrifuge the residual mixture.

The standard protocol allows the elution of the bound DNA fragment with standard volumes of Elution Buffer lower than that of the PCR sample introduced as sample, in order to result in very highly concentrated DNA. Minimum amount of Elution Buffer is 10 µl.
Repetition of elution (final step) may enhance recovery. In the vast majority of cases, however, up to 90 % of the elutable DNA is recovered during the first elution step.

For centrifugation we recommend a standard microcentrifuge. Centrifugation steps should be performed at room temperature.

Before start, be sure to

No steps necessary prior to preparation.

4. Workflow

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

1. DNA Binding	
Place one spin column into one 2 ml collection tube.	
Add 500 µl **Binding Buffer** to the spin column.	
Add up to 50 µl of the PCR reaction mixture to the Binding Buffer in the spin column reservoir. Mix by pipetting three times up and down.*	
Close the cap and centrifuge at 10.000 x g (ca. 12.000 rpm) for 2 mins/RT. Discard the flow-through and the collection tube.**	

Alternative DNA Binding	
Place one spin column into one 2 ml collection tube.	
Add 500 µl **Binding Buffer** to a 1.5 ml reaction tube.	
Add up to 50 µl of the PCR reaction mixture to the Binding Buffer. Mix by pipetting three times up and down.	
Apply this sample / Binding Buffer mixture completely into the spin column reservoir.	
Close the cap and centrifuge at 10.000 x g (ca. 12.000 rpm) for 2 mins/RT. Discard the flow-through and the collection tube.**	

2. Elution	
Make sure the spin column is dry and didn't have contact with the flow through after centrifugation. If not, repeat centrifugation and discard the tube.***	
Place the Spin Column into a clean 1.5 ml elution tube.	
Add 10-50 µl **Elution Buffer EB** to the centre of the membrane.****	
Incubate for 1 min at room temperature.*****	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute DNA.	

* Don't touch and destroy the membrane in the process.
** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.
*** Residual Binding Buffer may reduce recovery of the DNA.
**** Elution with lower volumes of Elution Buffer increases the final concentration of DNA. Minimum amount of Elution Buffer is 10 µl.
***** In order to increase the amount of recovered DNA, elution incubation time may be enhanced to 5 mins. However, the vast majority of DNA is eluted during the first minute.

Store the extracted DNA at +4 °C. For long time storage placing at -20 °C is recommended.

5. Trouble Shooting

Problem / probable cause	Comments and suggestions
Low recovery	
Poor elution of DNA.	Add the Elution Buffer directly onto the centre of the Spin Column.
	Apply the correct centrifugation steps.
	Prolong elution time up to 5 mins.
	Heat the elution buffer to 45 °C prior to elution.
	Check PCR / amplicon concentration prior to elution.
Problems with down-stream application, e.g. ligation,	
Ends of PCR fragments	Ends not correct. No TA-addition, no blunting, restriction site incorrect. Check PCR conditions of conditions of modifying processes.
Interference of mineral oil	Increase volume of Binding Buffer 2fold.
Interfering residues in eluted DNA	Increase volume of Binding Buffer 2fold.
	Prolong centrifugation time and check for residual Buffer /wet spin column after centrifugation.

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

ROTI®Prep PCR Purification	**10 preps (Mini kit)**	**8503.1**
ROTI®Prep PCR Purification	**50 preps (Kit)**	**8503.2**
ROTI®Prep PCR Purification	**250 preps (Maxi kit)**	**8503.3**

Carl Roth GmbH + Co. KG
Schoemperlenstraße 3-5 • 76185 Karlsruhe
P.O. Box 100121 • 76231 Karlsruhe
Phone: +49 (0) 721/ 5606-0
Fax: +49 (0) 721/ 5606-149
info@carlroth.com • www.carlroth.com
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