Instructions for use

ROTI®Prep Gel Extraction

8510

For DNA extraction from agarose gels

Introduction and product description:

- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Extraction within approx. 20 minutes
- For fragment length of 100 bp-30 kb
- Recovery rate up to 90 %

DNA extraction from agarose gels through incubation and subsequent spin column process. The gel slice to be extracted (up to 300 mg) is first solubilised in a lysis buffer through a simple 10-minute incubation.

After that, DNA is eluted via convenient spin filter centrifugation in approx. 10 mins. A specially developed Binding Enhancer is admixed in order to enhance binding efficiency of the DNA to the filter, and thus yield and purity of the isolated DNA.

Furthermore, the ROTI®Prep Gel Extraction kit allows, if needed, to reduce elution volume down to 10 µl in order to obtain particularly highly concentrated DNA.

For gel runs both, TAE and TBE may be used. The kit is compatible with all standard gel loading buffers and gel staining reagents, even non-toxic ethidium bromide replacements like, for instance, ROTI®GelStain (Art. Nr. 3865).

Isolated DNA is free of residual agarose, PCR- and ligase inhibitors, and cytotoxic reagents, and can directly be applied to all standard downstream applications like cloning, PCR, transformation and transfection.

For research use only.

Information in this document is subject to change without notice.
Carl Roth GmbH + Co. KG assumes no responsibility for any errors that may appear in this document.
Carl Roth GmbH + Co. KG disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall Carl Roth GmbH + Co. KG be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

Caution:

Gel Solubiliser **Danger** H302+312+H332-H314-H412-EUH032

Binding Enhancer **Warning** H315-H319

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 Gel Extraction Kit*.

1. Materials provided with the kit and storage conditions

Amount	Component	Storage*
5 / 5 / 15 ml	Binding Enhancer	RT
2 / 2x2 / 15 ml	Elution Buffer EB	RT
7 / 40 / 180 ml	Gel Solubilizer*	RT
6 / 16 / 2x36 ml	Washing Solution WSL (Base)	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 gel solubiliser is now filled in a white bottle.

The ROTI®Prep Gel Extraction 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 up to 1 year 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 Extraction 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

- Ethanol 96-99.8 %, e.g. Ethanol p.a. (e.g. 9065.1)

3. Application

ROTI®Prep Gel Extraction Kit is designed for isolation of high-purity DNA from agarose gels (TAE or TBE) 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 90 % DNA per preparation from maximally 300 mg slices of agarose gel. We recommend to minimize the agarose gel slice. Do not use more than 300 mg gel slice for one column!

The standard protocol allows the elution of the bound DNA fragment with standard volumes of Elution Buffer (30-50 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. Elution with lower volumes of Elution Buffer increases the final concentration of DNA. However, Elution in 10-20 µl Elution Buffer only reduces the recovery rate by approx. 10 %. We recommend to heat the needed amount of Elution Buffer to 50°C, which will increase the DNA yield.

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

Before start, be sure to

- Add Ethanol (96-99.8 %, not incl. in this kit) to the Washing Solution WSL as follows:
  8510.1 (10 Preps): 24 ml (30 ml final vol.)
  8510.2 (50 Preps): 64 ml (80 ml final vol.)
  8510.3 (250 Preps): 144 ml to each bottle (2x 180 ml final vol.)
  Mix thoroughly and keep the bottle always firmly closed!
- Heat a thermal mixer or water bath to 50 °C
- Optionally: heat the needed amount of Elution Buffer to 50°C. The final elution step with heated Elution Buffer will increase the DNA yield!
  Please note: Centrifugation steps should be carried out at room temperature

4. Workflow

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

1. Dissolving of the agarose	
Excise the DNA fragment from the agarose gel with a sharp scalpel.	
Minimize the agarose gel slice to 300 mg in maximum.*	
Transfer the gel slice into a 1.5 ml or 2.0 ml reaction tube and add 650 µl **Gel Solubilizer**	
Incubate for 10 min at 50 °C until the agarose gel slice is completely dissolved.**	

2. DNA Binding	
Add 50 µl **Binding Enhancer** and mix the suspension by vortexing or pipetting.	
Apply the sample onto a Spin Column (green) placed on top of a 2.0 ml collection tube.	
Close the cap and centrifuge at 10.000 x g (ca. 12.000 rpm) for 1 min/RT. Discard the flow-through and reuse the collection tube.***	

3. Column Washing	
Add 700 µl of **Washing Solution WSL** to the Spin Column	
Close the cap and centrifuge at 10.000 x g (ca. 12.000 rpm) for 1 min/RT. Discard the flow-through and reuse the collection tube.***	
Add 700 µl of **Washing Solution WSL** to the Spin Column	
Close the cap and centrifuge at 10.000 x g (ca. 12.000 rpm) for 1 min/RT. Discard the flow-through and reuse the collection tube.***	
Centrifuge empty columns at 10.000 g (ca. 12.000 rpm) for approx. 2 mins/RT in order to remove residual ethanol.	

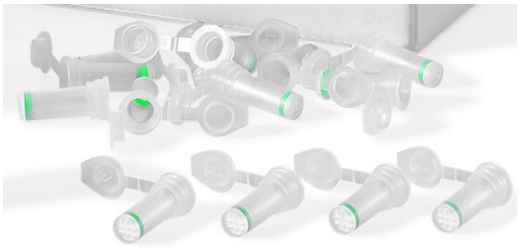
6. Elution	
Place the Spin Column into a clean 1.5 ml elution tube.	
Add 30-50 µl **Elution Buffer** (optionally pre-warmed to 50 °C) to the centre of the membrane.****	
Incubate for 1 min at room temperature	
Centrifuge at 6.000 g (or 8.000 rpm) for 1 min/RT to elute DNA.	
Optional: Elution may be repeated with 30 µl Elution Buffer *****	

* Try to keep the UV light applied to the gel slice at a minimum. UV light might damage DNA and, therefore, reduce the clonability of the eluted DNA.
** We recommend to use 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 incubation.
*** If the solution has not completely passed through the spin column, centrifuge again at higher speed or prolong the centrifugation time.
**** Elution with lower volumes of Elution Buffer increases the final concentration of DNA. In case DNA shall be eluted in 10-20 µl only, preheating of the elution buffer is obligatory. Using heated Elution Buffer (approx. 50 °C) will increase the DNA yield
***** Repetition of elution may enhance recovery.

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	
Incorrect Washing Solution or no ethanol added.	Prepare the Washing Solution exactly as described in the manual.
	Store the Washing Solution with firmly fixed cap.
Poor elution of DNA.	Add the Elution Buffer directly onto the centre of the Spin Column.
Ineffective solubilization of the agarose gel slice.	Extend the time used for dissolving of the agarose slice. Mix throughout this process a few times by vortexting or pipetting.
No Binding Enhancer added.	Add the required amount of Binding Enhancer to the solubilized suspension.
Problems with down-stream application, e.g. ligation,	
Contamination with salt components.	Wash the Spin Column as described in the manual.
Contamination with agarose traces.	Wash the Spin Column once with Gel Solubilizer.
Contamination of the final DNA with ethanol.	Make sure to centrifuge according to the manual. Extend centrifugal time if necessary. If centrifuging with lower rpm/g, longer time has to be applied.
DNA degraded.	Minimise the effect of UV light applied to the DNA by accelerate the cutting of the agarose gel slice and/or shifting to longer wave length (360 nm).



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

ROTI®Prep Gel Extraction	**10 preps (Mini kit)**	**8510.1**
ROTI®Prep Gel Extraction	**50 preps (Kit)**	**8510.2**
ROTI®Prep Gel Extraction	**250 preps (Maxi kit)**	**8510.3**

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
sse 06/2021