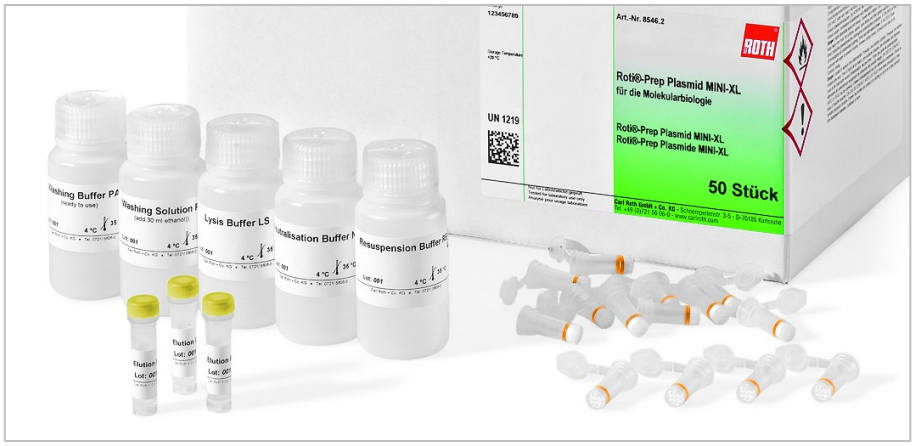
Instructions for use

ROTI®Prep Plasmid MINI-XL

8546

For easy isolation of plasmid DNA from up to 15 ml bacterial culture



Introduction and product description:

- Preparation by well-known mini spin-column system
- Fast, easy and efficient
- Preparation from 0.5-15 ml bacteria culture
- Preparation within approx. 20 minutes
- Yield: Mini-Prep (2.0 ml) up to 20 µg, Mini-XL-Prep (15 ml) up to 80 µg

ROTI®Prep Plasmid MINI-XL is a further development of the classical Plasmid Mini extraction kit (Ord. No. HP29), which is based on an alkaline lysis and binding of the plasmid to the spin filter.

Compared to usual plasmid mini kits, the binding capacity of the spin filter has been considerably increased (approx. 80 µg plasmid DNA), now allowing plasmid extraction from 0.5-15 ml bacteria culture. Since filter dimensions correspond to the usual miniprep spin filters, its application, however, differs only slightly from other plasmid mini kits. Most significant difference is the afore done convenient lysis in a 15 ml tube.

The elution, resulting in highly concentrated pure DNA in 100 µl, takes then place in a 1.5 ml tube, like in standard mini kits. Isolated DNA is free of RNA or protein contamination and can be directly applied to all standard down-stream applications like restriction digest, sequencing, *in vitro* transcription, transfection etc.

For research use only.

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: Please observe the information on the label and the safety data sheet.

*Lysis Buffer LS**	**Danger** H315-H318
Neutralisation Buffer NB	**Warning** H302-H315-H319
Washing Buffer PA	**Danger** H225-H302-H315-H319-H336

*In case of precipitation of the SDS, please dissolve by careful warming.

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 Plasmid MINI-XL Kit*.

1. Materials provided with the kit and storage conditions

Amount	Component	Storage
12 / 30 / 150 ml	Resuspension Buffer RB incl. RNase A [0.2 mg/ml]*	RT / +4-8 °C
15 / 30 / 150 ml	Lysis Buffer LS	>20 °C**
12 / 2x32 / 200 ml	Neutralisation Buffer NB	RT
15 / 2x30 / 200 ml	Washing Buffer PA (ready-to-use)***	RT
6 / 20 / 80 ml	Washing Solution PB (Base)	RT
2 / 15 / 30 ml	Elution Buffer XEB	RT
10 / 50 / 250	Mini spin columns (orange)****	RT
10 / 50 / 250	1.5 ml Elution tubes	RT
10 / 50 / 250	2 ml Collection tubes	RT

* Resuspension Buffer already contains RNase A. The buffer may well be stored at room temperature for a few weeks. For longer periods, store Resuspension Buffer RB cooled to approx. +4 to +8 °C.

** Lysis Buffer should be stored at over 20 °C in order to prevent SDS precipitation.

*** Washing Buffer PA is ready-to-us. No ethanol has to be added.

**** Colour of the spin columns has been altered from black to orange.
No other parameter has been changed.

The ROTI®Prep Plasmid MINI-XL 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 Plasmid MINI-XL 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)
- 15 ml reaction tubes for cell lysis

3. Application

ROTI®Prep-Plasmid MINI-XL is designed for isolation of high-purity plasmid or cosmid DNA from prokaryotic cells for subsequent sequencing, restriction digests, or transformations. 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 80 µg** DNA per preparation from maximally 15 ml bacterial culture, while still being performed in **standard MINI spin columns** and lab bench centrifuges. Typical yields of plasmid DNA are 40-60 µg on average if 15 ml culture medium saturated with bacteria carrying high-copy plasmids are used for isolation.

The RNase A is already solubilized in the Resuspension Buffer and hasn't to be added. During lysis and neutralization don't vortex the tube to mix the suspension! This step is critical for the separation of bacterial chromosomal DNA from plasmid DNA. Mechanical stress by vortexing or extensive mixing leads to shearing of high-molecular weight chromosomal DNA. This sheared chromosomal DNA is not precipitated by NaOH/SDS and contaminates the plasmid DNA.

Repetition of elution (final step) may enhance recovery. In the vast majority of cases, however, 95 % of the elutable DNA is recovered during the first elution step.

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

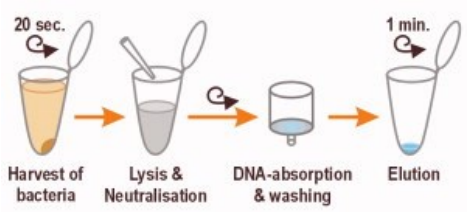
For centrifugation we recommend a standard microcentrifuge.
Centrifugation steps should be carried out at room temperature.

Before start, be sure to

- Add Ethanol (96-99.8 %, not incl. in this kit) to the Washing Solution PB as follows:
  8546.1 (10 Preps): 9 ml (15 ml final vol.)
  8546.2 (50 Preps): 30 ml (50 ml final vol.)
  8546.3 (250 Preps): 120 ml (200 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

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. There are twoe separate protocols. One for extraction from 0.5-5 ml bacterial culture and one for Plasmid-DNA-extraction from 5-15 ml bacterial culture



1. Isolation from 0.5-5 ml bacterial culture

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

1. Cell Harvest and Resuspension	
Harvest the bacterial cell culture (0.5-5 ml) by centrifugation for 1 min at full speed. Discard supernatant and remove remaining culture medium carefully.	
Resuspend pelleted bacterial cells completely in 250 µl **Resuspension Buffer RB** (incl. RNase A)	

2. Cell Lysis and Neutralization	
Add 250 µl of **Lysis Buffer LS**. Mix gently by inverting the tube 10 times (Do not vortex!)	
Incubate for 5 mins at room temperature (not longer!)	
Add 350 µl of **Neutralization Buffer NB** and **immediately** mix by inverting the tube 10x until a homogenous solution is achieved	
Centrifuge at 12.000-14.000 rpm (or full speed) for 8 mins/RT	

3. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the **clearified supernatant from step 2** into the spin column. Be sure to omit as much of the white precipitant as possible.	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through*	
Repeat the centrifugation step or centrifuge at higher speed if the solution has not completely passed the filter membrane	

4. Column Washing	
Add 500 µl of **Washing Buffer PA** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through*	
Add 700 µl of **Washing Solution PB** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through*	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 2 mins/RT in order to remove residual ethanol.	

5. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 50-100 µl **Elution Buffer XEB** or sterile, distilled water to the centre of the membrane**	
Incubate for 3 mins at room temperature	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT to elute DNA	

* 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 50 µl only, preheating of the elution buffer is obligatory. Using heated Elution Buffer (approx. 50 °C) will increase the DNA yield. Alternatively, DNA may be eluted in 2x 50 µl buffer. If so, use 50 µl preheated buffer for elution, centrifuge. Then repeat this elution process with another 50 µl and finally pool both fractions.

1. Isolation from 5-15 ml bacterial culture

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

1. Cell Harvest and Resuspension	
Harvest the bacterial cell culture (5-15 ml) by centrifugation for 10 mins at full speed. Discard supernatant and remove remaining culture medium carefully.	
Resuspend pelleted bacterial cells completely in 550 µl **Resuspension Buffer RB** (incl. RNase A)	

2. Cell Lysis and Neutralization	
Add 550 µl of **Lysis Buffer LS**. Mix gently by inverting the tube 10 times (Do not vortex!)	
Incubate for 5 mins at room temperature (not longer!)	
Add 750 µl of **Neutralization Buffer NB** and **immediately** mix by inverting the tube 10x until a homogenous solution is achieved	
Centrifuge at 12.000-14.000 rpm (or full speed) for 8 mins/RT	

3. Column Loading	
Place each Spin Column into a 2 ml collection tube	
Apply the **clearified supernatant from step 2** into the spin column. Be sure to omit as much of the white precipitant as possible.	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through*	
Apply the residual clearified supernatant from step 2 into the spin column. Be sure to omit as much of the white precipitant as possible.	
Centrifuge at 10.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through*	

4. Column Washing	
Add 650 µl of **Washing Buffer PA** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through*	
Add 750 µl of **Washing Solution PB** to the Spin Column	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT and discard the flow-through*	
Again centrifuge columns at 12.000-14.000 rpm (or full speed) for 2 mins/RT in order to remove residual ethanol.	

5. Elution	
Place the Spin Column into a clean 1.5 ml elution tube	
Add 100 µl **Elution Buffer XEB** or sterile, distilled water to the centre of the membrane**	
Incubate for 3 mins at room temperature	
Centrifuge at 11.000 g (or 12.000 rpm) for 1 min/RT to elute DNA	

* 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 50 µl only, preheating of the elution buffer is obligatory. Using heated Elution Buffer (approx. 50 °C) will increase the DNA yield. Alternatively, DNA may be eluted in 2x 50 µl buffer. If so, use 50 µl preheated buffer for elution, centrifuge. Then repeat this elution process with another 50 µl and finally pool both fractions.

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 resuspension or lysis of bacteria	The bacterial pellet has to be resuspended completely. Make sure there is no culture medium present before adding the resuspension buffer.
Inefficient neutralization.	Do not shake or vortex the sample after adding Neutralization Buffer. Mix by inverting the tube 10 times.
	The suspension has to be completely homogeneous after mixing.
Bacteria overgrown	Overgrowth of bacteria during over night culture may lead to reduction of the antibiotic present (usually ampicillin) and thus to growth of non-recombinant bacteria. Enhance dose of antibiotics to 200 µg/ml and reduce growth period to 8 hours.
Background protein expression	High-copy production of plasmids may lead to background expression of the inserted proteins. Presence of these proteins in bacteria may be cell-toxic, even if the protein itself is not, leading to low propagation rates, overgrowth by non-recombinant bacteria or elimination of the plasmids.
Problems with down-stream application, e.g. ligation,	
Contamination with salt components.	Wash the Spin Column as described in the manual.
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.

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

ROTI®Prep Plasmid MINI-XL	**10 preps (Mini kit)**	**8546.1**
ROTI®Prep Plasmid MINI-XL	**50 preps (Kit)**	**8546.2**
ROTI®Prep Plasmid MINI-XL	**250 preps (Maxi kit)**	**8546.3**

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
lha 06/2023