Ron's Plasmid Mini Kit

Kit for the purification of plasmid DNA from *E. coli* and other bacteria

Research Use Only (RUO)

Contents

Ron's Plasmid Mini Kit	Ref. No: 806942 (50 preps)
	Ref. No: 806942L (5 x 50 preps)
Valid from:	August 2019

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Ron's Plasmid Mini Kit

1. Introduction

The **Ron's Plasmid Mini Kit** presents remarkable features of timesaving, easy, prompt and low cost plasmid DNA purification from *E. coli* and other bacteria.
With Rons's Plasmid Mini Kit low copy number plasmids and high molecular size plasmids, including vectors like cosmids, can be isolated easily for subsequent analytical applications.
Plasmid DNA purified by the Ron's Plasmid Mini kit is ready for use for a broad panel of downstream applications:

- PCR
- Restriction enzyme digestion
- Labeling
- Ligation
- Transformation
- Transfection
- Sequencing
- In vitro transcription

2. Content of the Kit

Ref No	**S806942** 10 preps (sample size)	**806942** 50 preps	**806942L** 5 x 50 preps	**Storage**
Mini spin columns	10	50	5 x 50	RT
Collection tubes 2.0 ml	10	50	5 x 50	RT
Resuspension Buffer PR-1*	11 ml	11 ml	5 x 11 ml	2 - 8°C*
Lysis Buffer PL-2	11 ml	11 ml	5 x 11 ml	RT
Neutralization Buffer PN-3	25 ml	25 ml	5 x 25 ml	RT
Wash Buffer concentrate WB-2 **	5 ml	2 x 5 ml	10 x 5 ml	RT
Elution Buffer EL-3	6 ml	6 ml	5 x 6 ml	RT
RNase A	0.5 ml	0.5 ml	5 x 0.5 ml	-20°C
Manual	1	1	1	-

RT: Room temperature

* Add RNAse A to Resuspension Buffer PR-1. See manual page 4. Without RNase, PR-1 can be stored at RT.
** Add ethanol, see manual page 4.

3. Storage Conditions and Stability

Guarantee for full performance of the kit as specified in this manual is only valid if storage conditions are followed.
Store RNase A at –20 °C until use. RNase A can be stored under this condition for at least 1 year.
Spin columns of the kit are packed in closed bags and show full performance in this condition at room temperature (18 - 25 °C) for at least 2 years. Store buffers at room temperature (18-25 °C).
Resuspension Buffer PR-1 must be stored at 2 – 8 °C if RNase A is added.
Precipitates in buffers should be dissolved by warming up to 37 °C. Close bottles immediately after use.

4. Quality Control

The performance of the **Ron's Plasmid Mini Kit** is monitored routinely on a lot-to-lot basis.

5. Safety Information

The following components of Ron's Plasmid Mini Kit contain hazardous contents. It is strongly recommended to wear a lab coat, disposable gloves and protective goggles when working with chemicals. More detailed information is available in the material safety data sheets, which can be requested from the manufacturer. There is no need of labeling harmful features with H & P phrases upon packing sizes of 125 ml or 125 g.

Caution: Do not add bleach or acidic solutions to the waste of sample preparation.

Component	Hazard content	GHS symbol		Hazard phrases	Precaution phrases
Neutralisation Buffer PN-3	Guanidine hydrochloride 36-50%		Warning	302, 319	280, 301+312 305+351+338, 330, 337+313

Hazard phrases	
H302	Harmful if swallowed
H319	Causes serious eye irritation

Precaution phrases	
P280	Wear protective gloves / eye protection
P301+312	If swallowed: call a poison center/doctor/ …/ if you well unwell
P305+351+338	If in eyes: rinse cautiously with clean water for several minutes. Remove contact lenses. Continue rinsing
P330	Rinse mouth
P337+313	If eye irritation persists: get medical advice/attention

6. Protocol for Plasmid DNA preparation

6a. Preparation of Buffers

Additional Material Required:

- 96-100 % ethanol
- Incubator/ heat shaker or water bath
- Microcentrifuge
- Receiver tubes (1.5 ml)

Before starting:
Resuspension Buffer PR-1 is ready for use **after addition of RNase A**. For this, add the contents of the RNase A vial to Resuspension Buffer PR-1 by pipetting, close the bottle and mix by vigorous shaking (add 500 µl RNase A to 11 ml Resuspension Buffer PR-1).
Store complete Resuspension Buffer PR-1 bottle in the refrigerator (4-12 °C) for up to 4 months. Alternatively pipette 10 µl of RNase A solution to 200 µl Resuspension Buffer PR-1 (one reaction).

Wash Buffer WB-2 is ready to use after addition of 21 ml ethanol p.a. (95-99.8 %).

Required incubators /water baths
Heat an incubator up to 70 °C. Heat Elution Buffer EL-3 up to 70 °C for elution (see step 12).

6b. Protocol

1. Transfer 1 - 6 ml of stationary culture (overnight grown) to a 1.5 ml micro tube. Centrifuge at ≥ 13000 rpm (approx. 10000 g) for 5 minutes to sediment cells. Discard the supernatant.
2. Resuspend cell pellet in **200 µl Resuspension Buffer PR-1** including RNase A by vigorous vortexing. Pipetting up and down may accelerate resuspension.

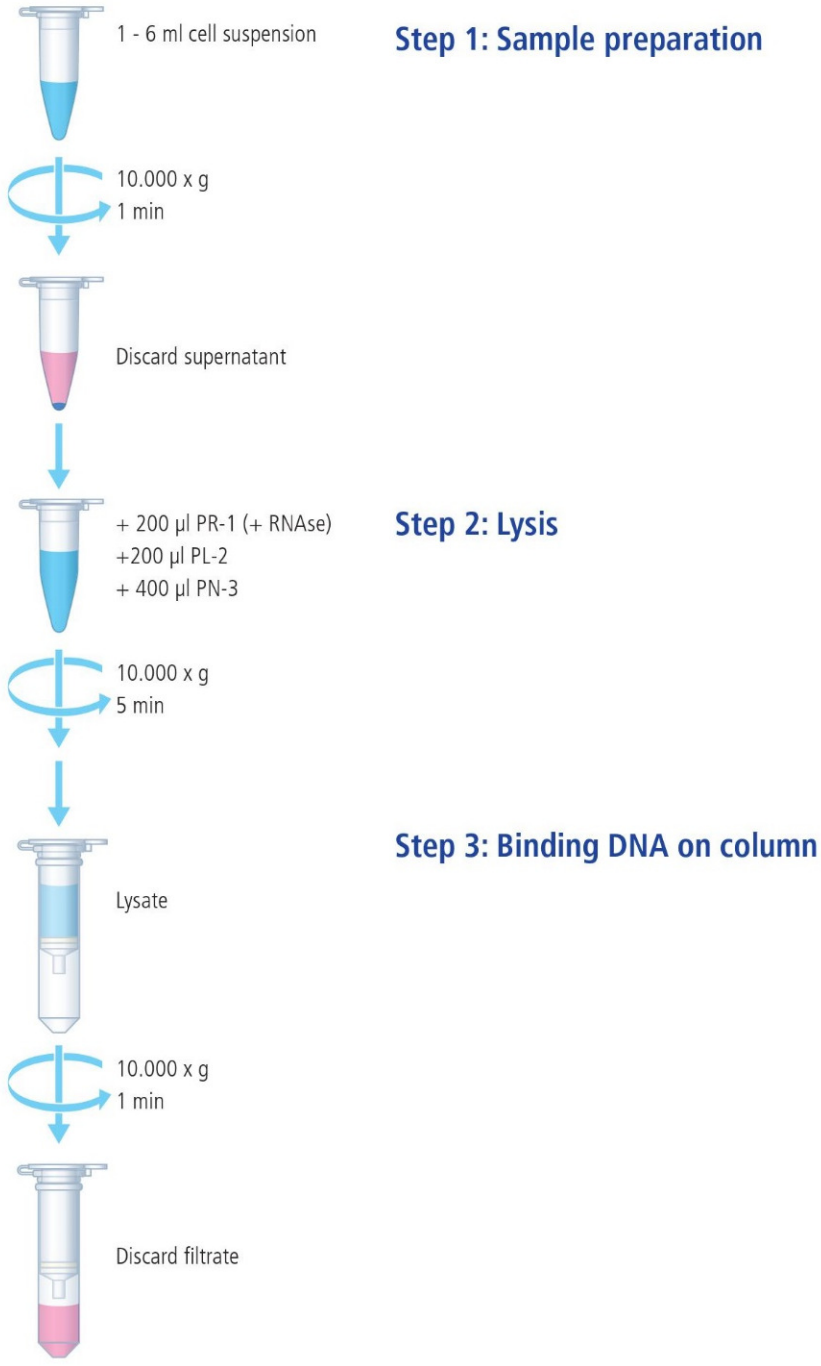
   Note: Make sure that small cell pellets or clumps are resuspended completely.
3. Add **200 µl Lysis Buffer PL-2** and mix carefully by inverting 4-6 times.

   (Note: Do not extend reaction longer than 5 minutes, because otherwise plasmid DNA may denature irreversibly).
4. Add **400 µl Neutralisation Buffer PN-3** and mix carefully by inverting 4-6 times. During neutralization denatured chromosomal DNA and protein are precipitated).
5. Centrifuge at ≥ 13000 rpm for 5 min. Put a mini spin column into a 2.0 ml collection tube.
6. Transfer the clarified plasmid-containing supernatant (do not transfer any of the white pellets) into the mini spin column and close the lid. Centrifuge at ≥ 13000 rpm for 1 min.
7. Add **500 µl Wash Buffer WB-2** (completed with ethanol) to the spin column. Centrifuge at ≥ 13000 rpm for 1 min.
8. Discard the filtrate (flow through). Put the mini spin column into the collection tube.
9. Add **500 µl Wash Buffer WB-2** into the spin column. Centrifuge at ≥ 13000 rpm for 2 min.
10. Discard the collection tube with filtrate.
11. Place the column in a 1.5 ml receiver tube (not provided) and heat the column at 70°C for 5 minutes to remove the rest of alcohols. Add **50-100 µl Elution Buffer EL-3** (heated at 70°C) to the column and incubate for 2 minutes at room temperature.
12. Centrifuge at 13000 rpm for 1 minute and collect the eluate and proceed with down-stream processing (gel electrophoresis, PCR etc.)

Note: it is very important to remove rests of alcohol. Please follow the instructions in steps 11 and 12.

Note: DNA yield depends on the elution volume. For highly concentrated plasmid DNA elute with 30 to 50 µl Elution Buffer EL-3 or deionized water. Elution can also be repeated with fresh Elution Buffer El-3 (e.g. 100 µl) to combine both eluates.

7. Flowchart of Extraction



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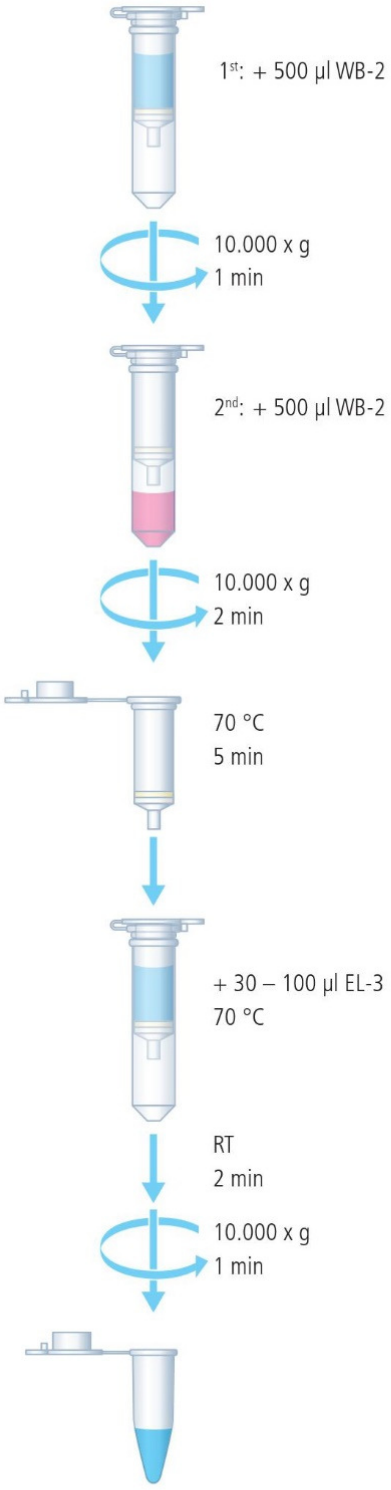

Step 4: Wash the column

Step 5: Elution of DNA

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8. Troubleshooting

This guide can help solve problems that may arise. BIORON GmbH welcomes comments and suggestions for improvement and supplement of our protocols or any hints on other molecular biology applications. The BIORON team is always pleased to answer any of your questions about our products. Please send us an email: info@bioron.net

Observation	Possible cause	Suggestions
No or low yield of plasmid DNA	Incorrect use of Wash buffer WB-2	Check if ethanol has been added
	Poor elution of DNA	Add the elution buffer directly to the center of the mini spin column.
	Plasmid segregation	Take care that growth has occurred in medium with the selective antibiotic.
Additional fragment below the supercoiled plasmid DNA band	Denaturated, supercoiled plasmid DNA	Increased incubation time with buffer PL-2 can cause irreversible denaturation of super-coiled plasmid DNA.
Contamination of the plasmid DNA with chromosomal DNA	Shearing of chromosomal DNA	Do not vortex after cell lysis. Also, invert tube carefully to keep shear forces at a minimum.

Inefficient lysis among other factors can decrease the yield of plasmid DNA. Attention should be paid also to the use of media that can influence the plasmid yield. We recommend LB medium (5 g yeast extract, 10 g peptone and 10 g NaCl per liter) for growth of *E. coli* and other heterotrophic bacteria.

9. Warranty and Guarantee of Products

The manufacturer guarantees the performance of its Ron's Plasmid Mini Kit in the manner described in this manual. It is up to the user to determine the suitability of Ron's Plasmid Mini Kit for its particular use. In case a product fails to perform due to any reason except misuse, the manufacturer will replace it without further charge or refund the purchase price. We reserve the right to change, alter or modify our Ron's Plasmid Mini Kit to enhance its performance and design. The manufacturer's terms and conditions are available on request.

10. Limitations of Product Use

The use of all products of **Ron's Purification Kits** is strictly limited to research purposes. They are not to be applied for any diagnostic, including human, or drug purposes.