Ron's PCR-Pure Mini Kit

Kit for the isolation of DNA

Research Use Only (RUO)

Contents

Ron's PCR Pure Mini Kit	Ref. No: 806493 (50 preps)
	Ref. No: 806493L (5 x 50 preps)
Valid from:	August 2019

Ron's PCR-Pure Mini Kit

1. Introduction

Ron's PCR-Pure Mini Kit provides an easy, safe and reliable method for isolation and purification of DNA obtained as PCR product.
The rapid and efficient procedure requires no expensive equipment and completely avoids the usage of toxic and hazardous reagents such as phenol or chloroform. The procedure is based on optimized buffers and the use of our specially designed **Ron's spin columns**. The advanced buffer system is optimized for efficient recovery of DNA and removal of contaminants. DNA is adsorbed to the Ron's spin membrane and all impurities are efficiently removed by washing. The pure DNA is directly eluted in a special buffer. The components of this kit are sufficient for processing samples of up to 100 µl.

2. Content of the Kit

Ref No	**S806493** 10 preps (sample size)	**806493** 50 preps	**806493L** 5 x 50 preps
Mini spin columns	10	50	5 x 50
Collection tubes 2.0 ml	10	50	5 x 50
Resuspension Buffer RB-1	4 ml	17 ml	5 x 17 ml
Wash Buffer concentrate WB-2 *	5 ml	2 x 5 ml	10 x 5 ml
Elution Buffer EL-3	1.5 ml	6 ml	5 x 6 ml
Manual	1	1	1

* Add ethanol, see page 3

3. Storage Conditions and Stability

All components of the **Ron's PCR-Pure Mini Kit** should be stored dry at room temperature (15 - 25 °C). Under these conditions, the kit shows full performance for at least 24 months. Guarantee for full performance of the kit as specified in this manual is only valid if storage conditions are followed.

4. Quality Control

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

5. Safety Information

The following components of **Ron's PCR Pure 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
Resuspension Buffer RB-1	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 DNA Purification

Additional Material Required:

- 96-100 % ethanol
- 70 % isopropanol
- Incubator/ heat shaker or water bath
- Microcentrifuge
- Receiver tubes (1.5 ml)

Ron's PCR-Pure Mini Kit

Before starting:
Wash Buffer WB-2 is a concentrate. Before using for the first time, add the appropriate amount of ethanol (96 - 100 %) as indicated on the bottle and in the table below:

Kit size	Wash Buffer WB-2	Ethanol to be added	Final volume
10, 50	5 ml	21 ml	26 ml

Procedure:

This protocol is designed for purification of single- or double stranded DNA fragments from up to 100 µl PCR-samples. For very small samples volumes < 30 µl adjust the volume of the reaction mixture to 100 µl with water.

1. Add **300 µl Resuspension Buffer RB-1** to the sample (100 µl) and mix with the vortexer.
2. Add **350 µl 70 % isopropanol** to the mixture and mix well.
3. Place a spin column in a provided 2 ml collection tube.
4. To **bind DNA,** apply the sample **(750 µl)** to the column and centrifuge for 30 - 60 s at 10000 g (approx. 13000 rpm).
5. Discard flow-through. Place the column back into the same tube. Collection tubes are re-used to reduce plastic waste.
6. **For washing**, add 500 µl **Wash Buffer WB-2** (add ethanol before use, as indicated on the bottle) to the column and centrifuge for 30 - 60 s at 10000 g.
7. **Second wash step**: add 500 µl **Wash Buffer WB-2** to the column and centrifuge for 2 min at 10000 g (approx. 13000 rpm).
8. Discard flow-through and place the column back in the same tube.
9. Place column in a clean 1.5 ml receiver tube (not included) and heat the column at 70°C for 5 min to remove the rest of alcohols.
10. For elution, add 30 µl - 50 µl **Elution Buffer EL-3** (pre-heated to 70°C) to centre of the column membrane**,** incubate at room temperature (18 - 25°C) **for 1 min** and centrifuge the column for 1 min at 10000 g to collect the eluate.
11. Incubate the open cup with the DNA eluate at 70°C for 5 minutes to remove the rest of the alcohol and proceed with down-stream processing (gel electrophoresis, PCR etc.)

7. Troubleshooting

This guide can help solve problems that may arise.

Observation	Possible cause	Suggestions
Poor or low recovery	Improper washing	Confirm the buffers were diluted with the specified volume of isopropanol and ethanol. Keep bottles tightly capped between uses to prevent evaporation.
	Poor elution	Repeat elution or increase elution volume.
Low A260/280 ratio	Purification is in-complete due to column overloading or inadequate lysis	If the system is overloaded, low yields and impure DNA are attributable. Increase the sample volume as necessary, but avoid overloading the column.
Low DNA performance	Salt in eluate	Make sure that you followed all washing steps of the procedure.

8. Warranty and Guarantee of Products

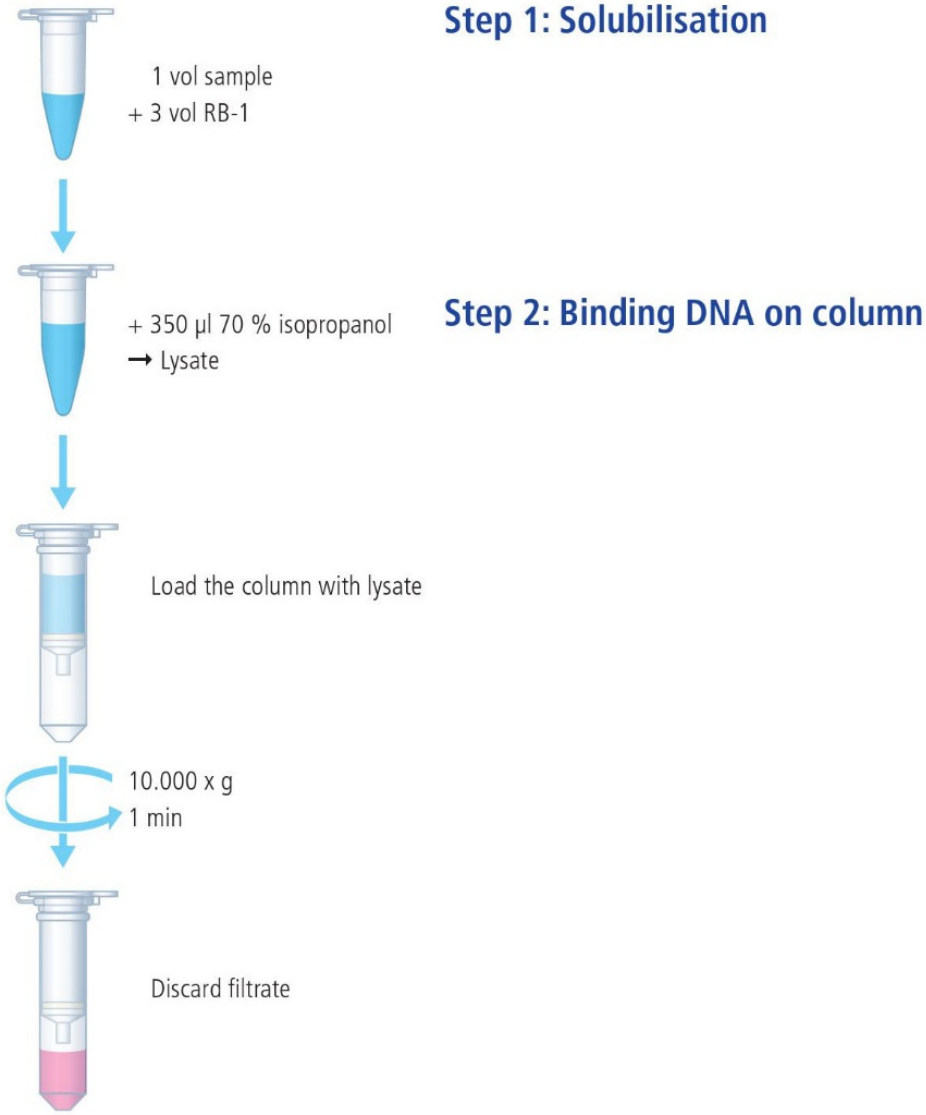
The manufacturer guarantees the performance of its Ron's PCR-Pure Mini Kit in the manner described in this handbook. It is up to the user to determine the suitability of Ron's PCR-Pure Mini Kit for its particular use. In case a product fails to perform due to any reason except misuse, the manufacturer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

9. Limitations of Product Use

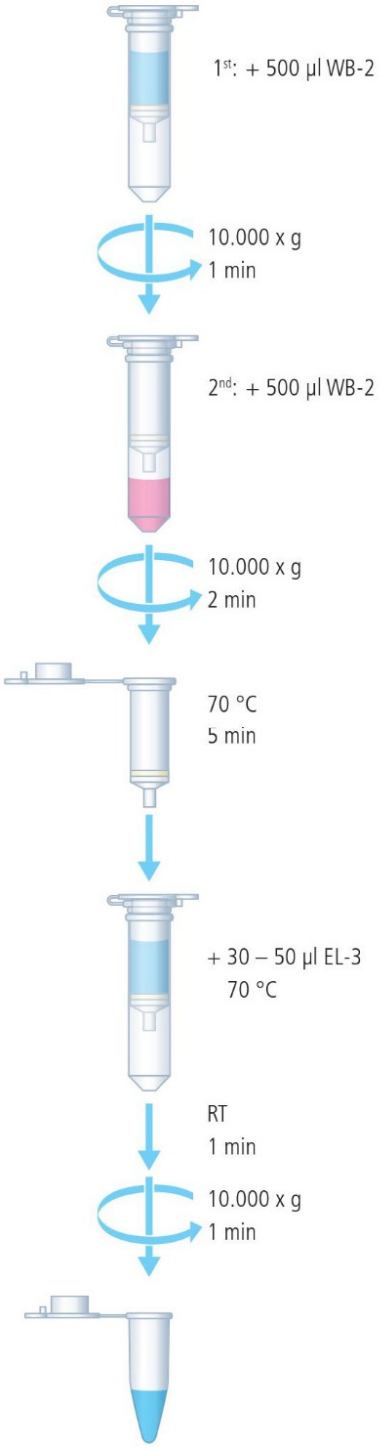
The use of all products of **Ron's PCR-Pure Mini Kits** is strictly limited to research purposes. They are not to be applied for any diagnostic use, including human or drug purposes.

Ron's PCR-Pure Mini Kit

10. Flowchart of Extraction



Ron's PCR-Pure Mini Kit



Step 3: Wash the column

Step 4: Elution of DNA