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## **PCR-Pure Kit**

PCR Purification based on silica technology

- MiniPrep

ROJETechnologies has been founded since 2014, and manufactures a wide range of molecular biology kits. We research, develop and create our products in order to make easier and more comfortable approaches to do research in molecular biology. Our target is offering high-quality affordable Molecular and diagnostic Kits and reagents, comparable of the world leaders, to research centers, laboratories, clinics, hospitals and diagnostic centers all over the world.

## Contents

Kit Content .....	4
Storage .....	4
Intended Use .....	4
Guarantee & Warranty .....	4
Notice to Purchaser .....	4
Warning and Precautions .....	5
Quality Control .....	5
Description .....	5
Equipment & Reagents to Be Supplied by User .....	5
Applications .....	5
Features .....	6
DNA Yield and Concentration .....	6
Buffer Preparation .....	6
Protocol: Purification of PCR product (based on silica technology) .....	7
Troubleshooting .....	9
Appendix 1. Yield and Purity of DNA .....	10
Appendix 2. Convert RPM to RCF (Centrifuge) .....	10
Ordering Information .....	10
Technical Assistance .....	10
Factory address .....	11

## Kit Content

Component	100 preps
<b>RPB</b>	12ml
<b>GWB</b>	10ml
<b>RSB</b>	5ml
<b>Micro column</b>	50
<b>Collection tube</b>	100

## Storage

Shipment condition is checked by ROJETechnologies. After arrival, all reagents should be kept dry at room temperature. When storage condition is as directed, all reagents are stable until expiration date, as indicated on the kit box.

## Intended Use

PCR-Pure Kit provides the components and procedures necessary for purifying genomic DNA and PCR product. Notice, PCR-Pure Kit is intended for molecular biology applications not for diagnostic use. We recommend all users to study DNA experiments guideline, before starting their work.

## Guarantee & Warranty

ROJETechnologies guarantees the efficiency of all manufactured kits and reagents. For more information on choosing proper kits based on your needs, please contact our technical support team. If any product does not satisfy you, due to reasons other than misuse, please contact our technical support team. If problem is due to manufacturing process, ROJE team will replace the Kit for you.

## Notice to Purchaser

This product is only for experiments and not for commercial use in any kind. No right to resell this kit or any components. For information about our licensing or distributors contact ROJE business team.

## Warning and Precautions

Due to chemical material usage that may be hazardous, always make sure to wear suitable lab coat, disposable gloves, and protective eyewear. Material Safety Data Sheet (MSDS) for all products and reagents are provided. They are accessible online at [www.rojetechnologies.com](http://www.rojetechnologies.com)

## Quality Control

PCR-Pure Kit is tested against predetermined experiments on a lot-to-lot basis according to ROJETechnologies ISO-certified quality management system, to ensure consistent product quality. For your information, the results of all experiments are accessible by addressing REF and Lot number on web at [www.rojetechnoloos.com](http://www.rojetechnoloos.com)

## Description

PCR-Pure Kit provides a rapid, precise and convenient method for efficient nucleic acid purification. The procedure is based on spin column technology, which takes less than 15 minutes. Special buffers provided with kit are enhanced for well-organized DNA recovery and contaminants removal. Due to presence of high concentration of salts, DNA adsorbs to the silica membrane and contaminants pass through the column. Finally, DNA is solved at prepared ROJE rehydration buffer. Typical yield, depends on density of starting material. The isolated nucleic acid is ready to use in downstream applications such as restriction endonuclease digestions.

## Equipment & Reagents to Be Supplied by User

- Absolute ethanol
- Pipets and pipet tips
- 1.5ml Microcentrifuge tube
- Vortex
- Microcentrifuge
- Dry Heat Block / Water Bath

## Applications

The isolated nucleic acid can be used in many downstream applications:

- Sequencing
- Restriction digestion

- Cloning

## Features

Specific features of PCR-Pure Kitara listed here in Table 1.

**Table 1.** PCR-Pure Kit features and specifications

Features	Specifications
Elution volume	30-50µl
Technology	Silica technology
Main sample type	PCR Product
Processing	Manual
Sample amount	Varies
Operation time per reaction	Less than 15min
Typical yield	15µg
Average purity	A260/A280= 1.7-2.0

## DNA Yield and Concentration

To obtain higher yield of DNA, it is important to follow protocol carefully. rehydration buffer amount, how the buffer is applied to the spin column, and the incubation time of rehydration buffer on the Micro Column effect on DNA yield.

**Note:** For less amount of rehydration buffer, it is recommended to add it exactly to the center of spin column.

## Buffer Preparation

Before the first use, add appropriate amount of ethanol (%96-100) to each washing buffer tube and RPB tube, then mix thoroughly to prepare buffers, refer to Table 2. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

**Table 2:** buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
GWB	10ml	40ml	50ml
RPB	12ml	28ml	40ml

## **Protocol: Purification of PCR product (based on silica technology)**

**Sample type:** PCR products

### **Some tips to know:**

- All centrifugation steps are carried out at room temperature (15–25°C).
- Don't forget to add the appropriate amount of molecular biology grade ethanol to RPB as indicated on the bottle, before using for the first time.
- Don't forget to add the appropriate amount of molecular biology grade ethanol (%96–100) to GWB as indicated on the bottle, before using for the first time (refer to buffer preparation section).
- RPB should be pink, if it turns yellow or orange, contact the technical support group.
- If RPB forms precipitate during storage, please warm it to 56°C until the precipitate has fully dissolved.
- RPB should be prepared. Not forget to add the appropriate amount of molecular biology grade ethanol (%96–100) to RPB as indicated on the bottle, before using for the first time (refer to buffer preparation section).

### **Process**

- Add 350 µl RPB to clean 1.5ml tube and 50-150 µl PCR Product then invert 10 times and 3s pulse vortex.
- Transfer the solution to a Micro Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 15s at room temperature.
- Discard the flow-through. Add 750µl GWB to the spin column. Centrifuge for 15s at 13000 rpm at room temperature. Discard the flow-through, and then centrifuge the Micro Column for an additional 1min at 14000 rpm.

**Note:** Discarding the flow-through before the second centrifuge is necessary to remove ethanol.



- Place the Micro Column in a new 1.5ml microcentrifuge tube. Add 30µl RSB directly to center of the spin column. Incubate at room temperature (15-25 °C) for 3 min. Centrifuge for 2 min at 14000 rpm to elute the nucleic acid.

## Troubleshooting

Here we try to cover as many problems as you may see in using this product, however scientists in ROJE technical support team are eager to answer all your questions. Do not hesitate to contact us for more information.

Symptoms	Problem	Suggestion
<b>Low nucleic acid recovery</b>	Using not prepared RPB	<ul style="list-style-type: none"> <li>Make sure to add absolute ethanol to RPB (%30 of total volume), before first use.</li> </ul>
	GWB did not contain Ethanol	<ul style="list-style-type: none"> <li>Not forget to add appropriate amount of ethanol to GWB, before first use.</li> </ul>
	Absence of PCR amplification	<ul style="list-style-type: none"> <li>Run the PCR product on gel before and after purification to make sure that the PCR amplification was done properly.</li> </ul>
<b>DNA does not perform well in downstream application</b>	Ethanol carryover	<ul style="list-style-type: none"> <li>Ensure that the traces of ethanol before rehydration step is removed</li> </ul>
	Salt concentration in elution	<ul style="list-style-type: none"> <li>After adding 750µl of GWB, incubate 5min at room temperature, and then centrifuge it.</li> </ul>
	Presence of Primer-primer dimer in DNA elution	<ul style="list-style-type: none"> <li>To completely remove primer-primer dimers, perform one additional step before adding GWB. Add 750µl of a %35-40 guanidine hydrochloride aqueous solution (35 g in 100 ml). Then continue the procedure by performing GWB to spin column.</li> </ul>
	Presence of ssDNA, appears as smear band on a gel electrophoresis	<p>Select one of these ways to reanneal the ssDNA:</p> <ul style="list-style-type: none"> <li>Incubate the mixture at 95°C for 3min then allow them to cool slowly at room temperature.</li> <li>Elute the DNA in 10mM Tris buffer containing 10mM NaCl.</li> </ul> <p><b>Note:</b> If using second method, consider, salt concentration for downstream application.</p>

## Ordering Information

Category	Product Name	Size	Cat NO
	PCR-Pure Kit	100 Preps	PG983004
	Gel and PCR Purification Set	100 Preps	PG983012
	GelJia Kit	100 Preps	PG983002

## Technical Assistance

ROJETechnologies guarantee your complete satisfaction. ROJE technical support team is composed of highly trained, experienced scientists; who can troubleshoot most problems you face. Our technical support team can offer expert advice which may help you select a suitable product.

- Contact our technical support at any time by selecting one of these ways:
- Through our telephone and fax number; +982191070705.
- You can submit your question directly to ROJE Technical Support Team from our website ([www.ROJETechnologies.com](http://www.ROJETechnologies.com))
- Or send your questions to this email address, [technicalsupport@rojetechnologies.com](mailto:technicalsupport@rojetechnologies.com).

## Appendix 1. Yield and Purity of DNA

The absorbance of DNA can be measured by any spectrophotometer. The ratio of absorbance at 260nm and 280nm is used to evaluate the purity of DNA. Pure DNA has an A260/A280 ratio of 1.8–1.9 and also a symmetric peak of absorbance at 260nm. If the ratio is lower in either case, it may indicate the presence of contamination. Proteins have absorbance at 280nm. EDTA, carbohydrate and phenol all have absorbance near 230nm.

## Appendix 2. Convert RPM to RCF (Centrifuge)

All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})(r)}}$$

Where **RCF**= required gravitational acceleration (relative centrifugal force in units of g); **r**= radius of the rotor in cm; and **RPM**= the number of revolutions per minute required to achieve the necessary g-force.

### **Factory address**

NO. 2 Farvardin street- Fernan Street- Tehran- Shahr Qods- Iran- Postal Code: 37531146130-  
phone: +982191070705



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