ROJETECHNOLOGIES

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PCR-Pure Kit

PCR Purification based on silica technology

- MiniPrep

By ROJE

Edition, 2020

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

Component	50 preps	100 preps
RPB	28 ml	2 x 28 ml
GWB	10 ml	2 x 10 ml
RSB	5 ml	10 ml
HiPure DR column	50	100
Collection tube	50	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.

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.rojetechnoloes.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
- 1.5 ml 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 Kitare listed here in Table 1.

Table 1. PCR-Pure Kitfeatures and specifications

Features	Specifications
Elution volume	30-50 µl
Technology	Silica technology
Main sample type	PCR Product
Processing	Manual
Sample amount	Varies
Operation time per reaction	Less than 15 min
Typical yield	15 µg
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 HiPure DR 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	10 ml	40 ml	50 ml
RPB	28 ml	12 ml	40 ml

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).
- Not forget to add the appropriate amount of molecular biology grade ethanol to RPB as indicated on the bottle, before using for the first time.
- Not 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

1. Transfer PCR product to a clean microcentrifuge tube. Add 5 volume of pre-prepared RPB to the tube.
2. Pulse vortex for 15-30 s. Transfer the solution to a HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1 min at room temperature.
3. Discard the flow-through. Add 750 µl GWB to the spin column. Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through, and then centrifuge the HiPure DR Column for an additional 1min at 13000 rpm.

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

4. Place the HiPure DR Column in a new 1.5 ml microcentrifuge tube. Add 30-50 µl RSB directly to center of the spin column. Incubate at room temperature (15-25℃) or at 60℃ for 5 min. Centrifuge for 1 min at 13000 rpm to elute the nucleic acid.

Note: If higher DNA yield is desirable, add another 30 µl RSB directly to the center of spin column. Incubate at room temperature (15-25℃) or at 60℃ for 5 min. Centrifuge for 1 min at 13000 rpm to elute the nucleic acid.

Note: Incubating RSB at 60℃ increases the yield but in some cases, it might lead to the presence of ssDNA.

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
Low nucleic acid recovery	Using not prepared RPB	• Make sure to add absolute ethanol to RPB (30% of total volume), before first use.
	GWB did not contain Ethanol	• Not forget to add appropriate amount of ethanol to GWB, before first use.
	Absence of PCR amplification	• Run the PCR product on gel before and after purification to make sure that the PCR amplification was done properly.
DNA does not perform well in downstream application	Ethanol carryover	• Ensure that the traces of ethanol before rehydration step is removed
	Salt concentration in elution	• After adding 750 µl of GWB, incubate 5 min at room temperature, and then centrifuge it.
	Presence of Primer-primer dimer in DNA elution	• To completely remove primer-primer dimers, perform one additional step before adding GWB. Add 750 µl of a 35-40% guanidine hydrochloride aqueous solution (35 g in 100 ml). Then continue the procedure by performing GWB to spin column.
	Presence of ssDNA, appears as smear band on a gel electrophoresis	Select one of these ways to reanneal the ssDNA: • Incubate the mixture at 95˚C for 3 min then allow them to cool slowly at room temperature. • Elute the DNA in 10 mM Tris buffer containing 10 mM NaCl.

	Note: If using second method, consider, salt concentration for downstream application.

Appendix 1. Yield and Purity of DNA

The absorbance of DNA can be measured by any spectrophotometer. The ratio of absorbance at 260 nm and 280 nm 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 260 nm. If the ratio is lower in either case, it may indicate the presence of contamination. Proteins have absorbance at 280 nm. EDTA, carbohydrate and phenol all have absorbance near 230 nm.

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.

Ordering Information

Category	Product Name	Size	Cat NO
PCR & Gel Purification	PCR-Pure Kit	50 Preps	PG983003
	PCR-Pure Kit	100 Preps	PG983004
	Gel and PCR Purification Set	50 Preps	PG983011
	Gel and PCR Purification Set	100 Preps	PG983012
	Safe PCR-Pure Kit	50 Preps	PG983015
	Safe PCR-Pure Kit	100 Preps	PG983016
	GelJia Kit	50 Preps	PG983001
	GelJia Kit	100 Preps	PG983002

Technical Assistance

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

Contact our technical support at any time by selecting one of these ways:

- Through our telephone and fax number available at ROJETechnologies website.
- You can submit your question directly to ROJE technical support team from our website (www.rojetechnologies. com).

Or send your questions to this email address,Technicalsupport@Rojetechnologies.com .

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