ROJETECHNOLOGIES

www.rojetechnologies.com

Gel and PCR Purification Set

PCR Purification and nucleic acid isolation from gel based on silica technology

- MiniPrep

By ROJE

Edition, 12/2022

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
Equipment & Reagents to Be Supplied by User 5
Applications 6
Features 6
Buffer Preparation 7
Protocol 1: Isolation of nucleic acid from agarose gel (based on silica technology) 7
Protocol 2: Purification of PCR product (based on silica technology) 8
Troubleshooting 10
Ordering Information 12
Technical Assistance 12
Appendix 1. Yield and Purity of DNA 12
Appendix 2. Convert RPM to RCF (Centrifuge) 12
Factory address 13

Kit Content

Component	100 preps
RGB	2 x 30 ml
RPB	12 ml
GWB	2 x 8 ml
RSB	20 ml
Micro Column	100
Collection tube	200

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

Gel and PCR Purification Set provides the components and procedures necessary for purifying genomic DNA, PCR product from gel and PCR product purification. Notice, Gel and PCR Purification Set 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

Gel and PCR Purification Set 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

Gel and PCR Purification Set provides a rapid, careful and convenient method for both efficient nucleic acid isolation from agarose gel and nucleic acid purification. The procedure is based on Micro Column technology, which takes less than 30 minutes for gel purification and less than 15 minutes for PCR purification. Special buffers provided with the 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 in prepared ROJE rehydration buffer. Typical yield depends on density of the bands on gel. 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
- Ethanol (96-100%)
- Pipets and pipet tips
- 1.5ml Microcentrifuge tube
- Vortex
- Centrifuge
- 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 Gel and PCR Purification Set are listed here in Table 1.

Table 1. Gel and PCR Purification Set features and specifications

Features	Specifications
Elution volume	30-50µl
Technology	Silica technology
Main sample type	DNA bands on agarose gel, PCR Product
Processing	Manual
Sample amount	• Up to 200mg agarose gel • For PCR product varied
Operation time per reaction	• Less than 30 min (for Gel Purification) • Less than 15 min (for PCR Product)
Typical yield	• Up to 10µg (for Gel Purification) • 15µg for PCR Product

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	8 ml	32 ml	40 ml
RPB	12 ml	28 ml	40 ml

Protocol 1: Isolation of nucleic acid from agarose gel (based on silica technology)

Sample type: DNA bands on agarose gel

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 (96–100%) to GWB as indicated on the bottle, before using for the first time (refer to buffer preparation section).
- RGB should be yellow, if it turns pink, set the pH to 5.0 by using sodium acetate (3M).
- If RGB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

- Remove the DNA fragment from the 1% agarose gel with a clean, sharp scalpel.
  Note: removing the extra agarose, and try to minimize the size of the gel slice.
- Weigh the gel slice in a colorless tube. Add appropriate RGB amount to the microcentrifuge tube (for each 100 mg of gel, add 300µl RGB. For example, if the gel weight is 200 mg add 600 µl RGB to the microcentrifuge tube).
- Pulse vortex for 5 s and incubate at 50°C for 10 min. During incubation, pulse vortex every 2 min.

- After dissolving the gel completely, add one gel volume of absolute ethanol to the microcentrifuge tube (for example for 30 mg gel, add 30 µl absolute ethanol), pulse vortex for 15 s.
- Transfer the solution to a Micro Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge for 1 min at 13000 rpm at room temperature. Discard the flow-through.
- Add 750 µl prepared GWB to the spin column.

  Optional: For increased DNA concentration, after adding GWB incubate at room temperature (15-25˚C) for 4 min.
- Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through, and then centrifuge the Micro Column for an additional 1 min at 13000 rpm.
- Place the Micro Column in a new 1.5ml microcentrifuge tube. Add 30µl RSB directly to the center of spin column; incubate at room temperature (15-25˚C) for 5 min. Centrifuge for 1 min at 13000 rpm to elute the nucleic acid.

Protocol 2: 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 it 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℃) 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
Low nucleic acid recovery	Incomplete solubilizing of gel slice	• It may be due to, large sample or inappropriate RGB amount. Repeat procedure, with smaller starting material and appropriate RGB amount.
	Too large gel slice	• The most recovery can be obtained from less than 200mg gel. However, for size bigger than 200mg, it is recommended to use multiple Micro Column.
	RGB turned yellow or orange	• RGB color should be yellow, however if color changes, set the pH at 5.00 by using H_2SO_4.
	GWB did not contain ethanol	• Please sure to add appropriate 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.
	Using not prepared RPB	• Make sure to add absolute ethanol to RPB (30% of total volume), before first use.
DNA does not perform well in	Ethanol carryover	• Ensure that the traces of ethanol before rehydration step is removed

downstream application	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 750µl of a 35-40% guanidine hydrochloride aqueous solution (35g in 100ml). Then continue the procedure by performing GWB to Micro 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.

Ordering Information

Category	Product Name	Size	Cat NO
	Gel and PCR Purification Set	100 Preps	PG983012
	PCR-Pure Kit	100 Preps	PG983004
	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; +982191070705.
- 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.

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

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.

Factory address:
No. 2, Farvardin St., Fernan St., Shahr-e-Qods, 3753146130, Tehran, IRAN.

Tel: 021 91070705