BIONEER

ISO 9001
Certified

AccuPrep® PCR Purification Kit

for 96 well vacuum block

Cat. No.: K-3034-2

User's Guide

Safety Warnings and Precautions

This kit is for research use only, and should not be used for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Always wear gloves when treating irritants or harmful reagents.

Warranty and Liability

All BIONEER products meet strict Quality Control standards, and are warranted to perform as described when used correctly. Problems should be reported immediately, and any liability incurred by BIONEER to the customer is limited to the replacement of the products. The customer must provide full details of the problem to BIONEER within 30 days, and return the product to BIONEER for examination.

Quality Management System ISO 9001 Certified

All aspects of our quality management system, from product development and production to quality assurance and supplier qualification, have been certified to meet world-class standards.

QC Testing

Each lot of BIONEER's product is tested in our quality control team as raw material prior to purchase. Acceptable lots are processed and tested again as finished product.
Prior to purchase, each lot of the product is tested by BIONEER's quality control team as raw materials. The acceptable lots are processed and retested as a finished product.

Trademarks

AccuPrep®96 PCR Purification Kit is trademark of Bioneer Corporation.

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AccuPrep® 96 PCR Purification Kit

Technical Manual

I. Description

Thank you for purchasing AccuPrep®96 PCR Purification Kit. This kit is designed for purification of 100bp to 10kb DNA from PCR reaction products within 30 minutes using standard 96 well format plate.

20bp to 40bp of oligouncleotide, commonly used as primer, and other PCR reaction materials, such as dNTP, polymerase, mineral oil and etc., can be easily removed with this kit.

Removal of mineral oil is unnecessary.

The principle of this kit's performance is based on the special feature of DNA that it is adsorbed in glass fiber under the specific conditions.

Amplified DNA through PCR reaction is selectively adsorbed to each column tube in 96 well, but protein, salts and other contaminants pass through without adsorption. DNA is isolated and eluted in the final elution step with the sterile diluted water and EL buffer (10mM Tris-Cl, pH 8.5)

Extracted DNA can be directly used in cloning, base sequencing, DNA microarray analysis and other biological applications without any additional purification.

II. Product contents

AccuPrep® 96 PCR Purification Kit is designed to purify 192 testing samples in the base of 20~50ul of PCR products and maintains its performance for at least a year.

***AccuPrep*®96 PCR Purification Kit**

Cat. No.	**K-3034-2**
PB Buffer	60 mL
Store at room temperature. *Caution : This buffer contains irritant chaotropic reagent.	
WB Buffer	2 X 50 mL
WB Buffer is stable for 1 year at room temperature. WB Buffer is supplied as concentrate and should be used with ethanol before use. *Caution : Cover the buffer to prevent ethanol vaporization.	
EL Buffer	30 mL
EL Buffer (10Mm Tris-Cl, pH 8.5) Store at room temperature.	
96 well plates and covers	
96 well binding plate	2 ea
96 well RV Plate	2 ea
96 well dome Plate	2 ea

III. Required reagents and equipments

1. 96 well vacuum block
2. Vacuum pump
3. Multi-Channel Pipette
4. Reservoir
5. Ethanol (96-100%)
6. 96 well vortexer (optional)

IV. Caution before Use

Before you proceed, the followings should be checked.

1.Add 200 mL of absolute ethanol to WB Buffer and mix them thoroughly.

2.Before the test, preheat the EL Buffer warm to 60□/140℉.

3.In order to use, a precipitation should be melted completely at 60□/140℉.

4.Start vacuum control of Vacuum block
New binding plate with a plate is equipped with a vacuum block
Measuring vacuum after pouring 100uL of dilute water into all well of binding plate.
Vacuum should be between –300 ~ -400mmHg.
High vacuum causes solution to be sputtered and low vacuum caused filtering time to be delayed.

□ After measuring vacuum value, plate is used with dry form.

V. Experimental Protocol

1.Assembled vacuum block is equipped with vacuum pump and 96 well binding plate.
Waste tray should be placed inside the vacuum block. When operating without waste tray, the vacuum pump will be damaged.

2.Add 5 volumes of PB Buffer to 1 volume of PCR reaction and mix well. If necessary, provided 96well dome plate can be used.
For instance, if 20ul of PCR reaction is used, use 100ul of PB Buffer accordingly. Removal of mineral oil is unnecessary. 96 well vortexer can be used. Be careful, excessive vortex time may cause DNA shearing.

3.Prepared mixture is transferred to 96 well binding plate.
At this time pre-moved mixture may be filtered and dropped to vacuum block waste tray while the mixture is transferred to 96 well binding plate.

4.Mixture should be passed through binding plate by operating vacuum pump. After mixture pass through, stop the operation of vacuum pump and wait until the pressure is released
Little space between vacuum block and 96-well plate causes non-vacuum situation even during vacuum's operation. When putting pressure from top to down, the gap between block and plate will disappear, and vacuum will occur.

5.Repeat step 4 above after putting WB Buffer 1mL(96X1ml) to 96 well binding plate column.

6.Wash again, with 1ml of WB Buffer (96x1mL)

7.After removing buffer in wash tray, grab 96 well binding plate firmly and shake to nozzle direction in order to get rid of rest buffer inside of column.

8.To dry 96 well binding plate, remove all in waste tray and operate vacuum pump for 10 minutes.

9.Raise 96 well binding plate slowly and check the dry condition to be white. When column is not dried completely, try to dry again by vacuum.
Closely examine four edges of 96 well binding plate to see if there is column filter that is not completely dried. Use provided silicon cover to increase vacuum for those column filter to be completely dried. However, DNA yield could be reduced if it is excessively dried. Ethanol in WB Buffer should be removed, because it interrupts enzyme reaction and causes to suspend sample in agaros gel electroporesis.
Also if there is any trace of ethanol in glass fiber, it lowers elution yield. Check if ethanol remains at upper-side wall of 96 well binding plate. Use oven or vacuum dryer to dry. This can remove ethanol completely but be careful not to over dry it, since it may cause to reduce DNA yield. Depending on the humidity of the air, the drying time can be adjusted.

10.Plate is placed on clean, 3M paper in order to eliminate the buffer of 96 well binding plate's nozzle.
DNA yield may be decreased or cross contamination may occur in case of mineral oil sticks to the nozzle. If necessary, clean it.

11.Remove waste tray by dismantling vacuum block.

12.Assemble vacuum block with 96 well dome plate instead of waste tray and 96 well binding plate is placed on the dome plate.

13.Leave the vacuum for 1~3 mins after pouring 80 uL of Buffer (96 x 80 uL) to 96 well binding plate column. Put EL Buffer on the center of filter.

14.Operate vacuum pump for 5 minutes for elution. Uniformal yield is obtained by selectively increasingvacuum with provided silicon cover.
The volume of buffer to be collected from DNA can be controlled

according to needs. Using less than 80 ul is optionally possible, however, DNA yield could be reduced due to the decreased volume. It will not cause any trouble to leave them longer or to use warm EL buffer, which is boiled up to 60℃ or 140℉. Over 7 0% DNA will be obtained through following this procedure. Especially the yield of DNA, bigger than 3kb, will be increased by leaving them for 10 minutes at 60℃ or 140℉. DNA dilution can increase EL Buffer volume for more yield.

Be careful when using other than EL Buffer buffer, because maximum DNA yield is achieved between Ph7.0~8.5.

When using sterile water, be careful to adjust pH and buffer containing EDTA, which do not affect to DNA yield but may affect enzyme reaction.

VI. Problem Solving

1. Low DNA yield or purity

1) Reduction of elution buffer causes low DNA yield. Using provided silicon cover under high vacuum can increase elution buffer yield.
2) Check the ratio of PCR product and PB Buffer –1:5 of mixture. Uncompleted mixture or improper ratio could decrease the DNA yield, may cause contamination and primer may not be removed.
3) Check if absolute ethanol is mixed to WB Buffer appropriately. Uncompleted mixture or improper ratio can make the DNA yield down and cause contamination and the primer may not be removed.
4) Elution buffer should be used. High salt concentration and inappropriate pH reduce the DNA yield. DNA yield can be increased by increaing temperature or controlling vacuum with reference of procedure.
5) Check if precipitation happened. In case of salt precipitation, the solution may not work properly.

2. DNA suspension state in electroporesis of agarose gel.

Some remained and collected ethanol may cause sample to be

suspended. Check if ethanol remains before users collect DNA or if 96-well binding plate is dry through centrifuge. Make sure that there is no remaining ethanol at the end of nozzle of 96 well binding plate. Solve the problems with the references of the procedure.

3. Enzyme reaction does not work well with extracted DNA

1) May decrease the concentration of salt with reference of procedure to protect the remaining salt. If necessary, clean one more time with WB Buffer.
2) Residual ethanol may be a major cause of these problems. Refer to the procedure and remove residual ethanol as possible.

4. Uneven DNA yield

1) Yield may differ depending on the size of DNA to be purified. After adding EL Buffer, yield can be increased by leaving the mixture for 5~10 mins at 60 degree.
2) This may be caused by uneven quantity of amplified DNA in PCR product. Check if quantity of loaded DNA is not excessive. Maximum capacity of each column in 96 well binding plate is less than 5~10ug.

3) The differences of volume of elution buffer collected from each column can be the major cause. Yield of elution buffer may be increased by high vacuum with provided silicon cover.

Reference

1. Boom, R. *et al.* (1990), *J Clin. Microbiol*., **28**, 495-503
2. Carter, M.J. and Milton, I.D. (1993) *Nucleic Acids Res*., **21**, 1044
3. Melzak, K. A. *et al*. (1996), *J Colloid and Interface Sci*., **181**, 635

VII. Ordering Information

Product	Content	Cat. No.
AccuPrep® PCR Purification Kit for 96 well Vacuum Block	96 x 2 Purification	K-3034-2
Individual components		
PB Buffer	60 mL	KB-1122
WB Buffer	50 mL	KB-1053
EL Buffer	30 mL	KB-1062
96 well RV Plates	2 ea	KA-0041
96 well dome Plates	2 ea	
96 well binding plates for PCR	2 ea	KA-0161

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