USER GUIDE

AccuPrep®
PCR/Gel Purification Kit

Cat. No. K-3037G

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AccuPrep® PCR/Gel Purification Kit

User Guide

K-3037G

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Please read all the information in booklet before using the unit

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Intended Use

AccuPrep® PCR/Gel Purification Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Safety Warning and Precaution

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information, please consult the appropriate Material Safety Data Sheet (MSDS).

Warranty and Liability

All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

Trademark

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Copyright

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Contents

Product Information

Components

Components	K-3037G (200 reactions)
FB Buffer (Fragment DNA Binding)	120 ml x 2 ea
PB Buffer (PCR Product Binding)	75 ml
WB2 Buffer (Washing)	16 ml x 3 ea
EA Buffer (Elution)	15 ml
BSTB Solution	36 ml
AccuPrep® Binding Column-II	200 ea
Collection Tube (Filtration)	200 ea
One Page Protocol	1 ea

Storage

The kit will maintain performance for at least two years under standard storage conditions. All buffers and binding columns can be stored at room temperature (15-25°C).

Specifications

AccuPrep® PCR/Gel Purification Kit	
Size Range	50 bp-20 kb
Turnaround Time	< 20 minutes (Gel Extraction) < 10 minutes (PCR Purification)
Column Binding Capacity	up to 30 μg
Elution Volume	30 μl
DNA Recovery	70-80% (Gel Extraction) 80-90% (PCR Purification)
DNA Purity	A_{260}/A_{280} > 1.8
Isolation Technology	Silica Column

*** Note:** There may be differences in measured values depending on the type of samples.

Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because FB Buffer and PB Buffer contain chaotropic salts which are irritants.
- Completely remove the protective seal in BSTB Solution. BSTB Solution may be discolored, but it does not affect nucleic acid extraction.
- Perform all centrifugation steps at room temperature.

Introduction

Product Description

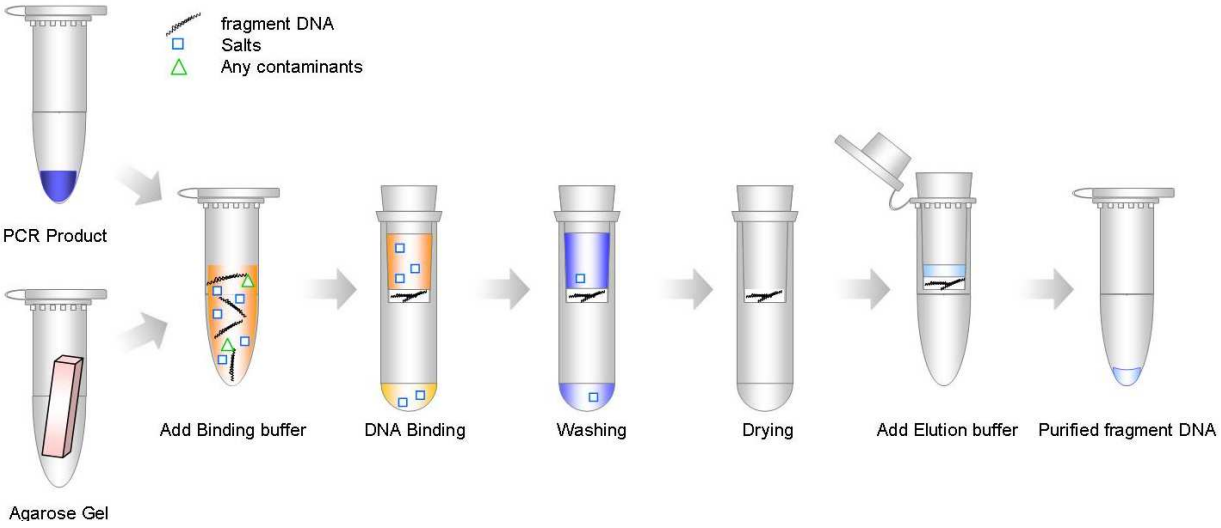
AccuPrep® PCR/Gel Purification Kit is designed for purification of up to 30 μg of DNA fragments from agarose gels or enzymatic reactions. The kit employs *AccuPrep*® Binding Column-II with silica membrane for nucleic acid binding in the presence of chaotropic salts. Chaotropic salts enhance not only dissolution of agarose gel but binding of DNA onto the silica membrane. Adsorption of DNA on the silica membrane is so selective that molten agarose and salts are not adsorbed and pass through the binding column. Any salts and residual agarose gels are eliminated through a series of short wash-and -spin steps using ethanol. Finally, highly purified DNA fragments are eluted in elution buffer or nuclease-free water. DNA fragments purified through this kit can be applied for a sub-cloning, sequencing and other molecular biological applications.

Features & Benefits

- Comprehensive: Highly can be purified DNA fragments from PCR products and various enzymatic reactants (restriction enzyme, A-tailing, Labeling reaction, etc.).
- Wide flexibility: Can be used on low-melting agarose gels, TAE, and TBE agarose gel.
- Efficient: Can lower the elution volume up to 30 µl.
- Ready-to-use: Extracted DNA fragment is ready-to-use for various application.

Experimental Procedures

Procedure Overview



Before You Begin

Before proceeding, please check the following:

1. Pre-heat EA Buffer at 60°C before use. This process is essential for maximal recovery in filter-based extraction.

2. Add indicated volume of isopropanol (not provided) to PB Buffer before use (see bottle label).

3. Add indicated volume of absolute ethanol (not provided) to WB2 Buffer and BSTB Solution before use (see bottle label).

PCR Purification

1. Add 5 volumes of PB Buffer to 1 volume of PCR reaction, then add 1 volume of isopropanol.
   * **Note:** If the PCR reaction is 20 µl, add 100 µl of PB Buffer, then 20 µl of isopropanol. Mix thoroughly by vortexing.

2. Add 100 µl of BSTB Solution to the *AccuPrep*® Binding Column-II fit in a Collection Tube.

3. Centrifuge at 13,000 rpm for 30 seconds. Discard the flow through. Reuse the Collection Tube in step 4.

4. Apply the mixture from step 1 to the *AccuPrep*® Binding Column-II fit.

5. Centrifuge at 14,000 rpm for 1 minute. Discard the flow through. Reuse the Collection Tube in step 6.

6. Wash the *AccuPrep*® Binding Column-II by adding 500 µl of WB2 Buffer.

7. Centrifuge at 14,000 rpm for 1 minute. Discard the flow through. Reuse the Collection Tube in step 8.

8. Wash the *AccuPrep*® Binding Column-II once more by adding 500 µl of WB2 Buffer.

9. Centrifuge at 14,000 rpm for 1 minute. Discard the flow through. Reuse the Collection Tube in step 10.

10. Centrifuge once more at 14,000 rpm for 1 minute to remove residual ethanol completely and check whether there is no droplet clinging to the bottom of the binding column.

11. Place the *AccuPrep*® Binding Column-II in a clean 1.5 ml tube (not provided). Add 30 µl of EA Buffer or nuclease-free water to elute DNA and let stand for 1 minute†. Centrifuge at 14,000 rpm for 1 minute.
    * **Note:** EA Buffer as well as TE buffer (pH 8.0) are suitable for ordinary downstream applications such as sequencing, restriction enzyme digestion, and ligation. On the contrary, elution of fragment DNA with pure water may cause the DNA denaturation and/or instability. TE buffer (pH8.0) can be used as EA Buffer except that EDTA may interrupt the subsequent enzymatic reactions.

† If DNA fragments are larger than 3 kb, increase incubation time to 10 minutes at 60°C.

12. To recover more DNA, repeat once more elution step using the eluate from step 11.

13. Centrifuge at 14,000 rpm for 1 minute.

Gel Purification

1. Excise the area of the agarose gel which contains desired DNA fragment as small as possible.

2. Weigh the gel slice in a clean 1.5 ml tube.
   * **Note:** The amount of gel slice should be less than 400 mg. If your gel slice exceeds 400 mg, use an additional binding column.

3. Add 3 volumes of FB Buffer to 1 volume of gel slice.
   * **Note:** If the amount of the gel slice is 200 mg, add 600 µl of FB Buffer.

4. Incubate the tube containing gel slice at 50˚C for 10 minutes. Invert the tube every 2-3 minutes for complete dissolution.
   * **Note:** The gel slice should be completely dissolved. If it has not melt completely, extend the incubation time.

5. After the gel slice appears dissolved, check the color† of the mixture from step 4 is yellow.
   * **Note:** If the color of the mixture is orange or red, add 10 µl of 3 M sodium acetate (pH 5.0) and mix so that the color should be turned into yellow.
   † Color of the mixture indicates pH of it. When a pH of the DNA fragment is less than 7.5 (yellow color), the fragment DNA can effectively bind to the binding column.

6. Add 1 volume of isopropanol to 1 volume of dissolved gel slice and mix gently.
   * **Note:** If the amount of the gel slice is 200 mg, add 200 µl of isopropanol.

7. Add 100 µl of BSTB Solution to the *AccuPrep*® Binding Column-II fit in a Collection Tube.

8. Centrifuge at 13,000 rpm for 30 seconds. Discard the flow through. Reuse the Collection Tube in step 9.

9. Apply the mixture from step 6 to the *AccuPrep*® Binding Column-II.

10. Centrifuge at 14,000 rpm for 1 minute. Discard the flow through. Reuse the Collection Tube in step 11.

11. Wash the *AccuPrep*® Binding Column-II by adding 500 µl of WB2 Buffer.

12. Centrifuge at 14,000 rpm for 1 minute. Discard the flow through. Reuse the Collection Tube in step 13.

13. Wash the *AccuPrep*® Binding Column-II once more by adding 500 µl of WB2 Buffer.

14. Centrifuge at 14,000 rpm for 1 minute. Discard the flow through. Reuse the Collection Tube in step 15.

15. Centrifuge once more at 14,000 rpm for 1 minute to remove residual ethanol completely and check whether there is no droplet clinging to the bottom of the binding column.

16. Place the *AccuPrep*® Binding Column-II in a clean 1.5 ml tube (not provided). Add 30 µl of EA Buffer or nuclease-free water to elute DNA and let stand for 1 minute†. Centrifuge at 14,000 rpm for 1 minute.

*** Note:** EA Buffer as well as TE buffer (pH 8.0) are suitable for ordinary downstream applications such as sequencing, restriction enzyme digestion, and ligation. On the contrary, elution of fragment DNA with pure water may cause the DNA denaturation and/or instability. TE buffer (pH8.0) can be used as EA Buffer except that EDTA may interrupt the subsequent enzymatic reactions.

† If DNA fragments are larger than 3 kb, increase incubation time to 10 minutes at 60˚C.

17. To recover more DNA, repeat once more elution step using the eluate from step 16.

18. Centrifuge at 14,000 rpm for 1 minute.

Troubleshooting

Problem	Comments
Low DNA yield	• **The gel slice may not have been completely dissolved.** Use an appropriate amount of gel slice, vortex frequently during incubation, and/or extend the incubation time.
	• **You may have used inadequate concentration of chaotropic salts, which affects DNA binding to binding column.** Use an appropriate volume of FB Buffer.
	• **There may have been incorrect pH in binding buffer.** Orange or red color in FB Buffer indicates that pH is out of range for adequate DNA binding reaction. Adjust pH of binding buffer with a 3 M sodium acetate (pH 5.0) (Refer to the no.5 of "Gel Purification" on page 8).
	• **Elution Buffer may have contained any salts.** Use EA Buffer included in this kit.
	• **Ethanol may not have been added to WB2 Buffer, BSTB Solution.** Add absolute ethanol (not provided) to the WB2 Buffer, BSTB Solution (see "Before You Begin" on page 5) and mix well. Mark WB2 Buffer, BSTB Solution bottle label to indicate whether ethanol has been added or not.
Sample floating upon loading in an agarose gel	• **Sample may have contained ethanol.** Floating is caused by remaining ethanol. Dry the column completely by centrifugation and make sure that no droplet is hanging from tip of the column.
Incomplete or no restriction enzyme cleavage of isolated DNA	• **Eluate may have contained high salt concentration.** Let the binding column stand for 5 minutes after adding WB2 Buffer, and then centrifuge.
	• **Sample may have contained residual WB2 Buffer.** Remaining ethanol interrupt the enzymatic reaction. The binding column should be dried completely. If the problem persists, let the binding column dry in the air for about 10 minutes after second centrifugation.

Ordering Information

Description		Cat. No
AccuPrep® PCR/Gel Purification Kit	200 reactions	K-3037G

Explanation of Symbols

LOT Batch Code	Consult Instructions For Use	RUO Research Use Only	Caution
Biological Risks	Contains Sufficient for <n> tests	Temperature Limitation	Manufacturer
REF Catalog Number	Do not Re-use	Use-by Date	

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