USER'S GUIDE

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AccuPrep®
PCR/Gel Purification Kit

REF K-3037
K-3038

BIONEER
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AccuPrep® PCR/Gel Purification Kit

User's Guide

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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.

Trademark

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1. Description

AccuPrep® PCR/Gel Purification Kit is designed for the purification of up to 30 μg of fragment DNA from agarose gel slice or from enzymatic products including PCR mixture. The size range for effective purification is about 50 bp–20 kb. The average recovery yield is between 70%–95%. Elution volume can be as little as 30 μl when concentrated product is needed. The principle of this kit is based on adsorption of DNA onto the silica-based membrane by chaotropic salt. Chaotropic salts enhance melting of agarose gel but binding of DNA onto the silica-based membrane that is located in a binding column tube. Adsorption of DNA on the membrane is so selective that molten agarose and salts are not adsorbed and pass through the binding column. Washing step eliminates salts and residual agarose gel. High-purity DNA fragments are eluted with provided elution buffer or distilled water. Purified DNA fragment can be applied to sub-cloning, sequencing and other molecular biological applications.

2. Kit components

	AccuPrep® PCR/Gel Purification Kit	
Cat. No	K-3037	K-3038
FB Buffer (Fragment DNA Binding Buffer)	120 ml x 2 ea	60 ml
PB Buffer (PCR Product Binding Buffer)	75 ml	20 ml
W2 Buffer (Washing Buffer)	80 ml × 3 ea	60 ml
EA Buffer (Elution Buffer)	15 ml	15 ml
BST Solution	40 ml	10 ml
AccuPrep® Binding Column-II	200 ea	50 ea
Collection Tube	200 ea	50 ea
One Page Protocol	1 ea	1 ea

※ FB Buffer and PB Buffer contain chaotropic salt and should be handled with care. Chaotropic salts can makes highly reactive compounds when mixed with disinfecting agent such as bleach.

※ All buffers and binding columns can be stored at room temperature.

Additional required materials

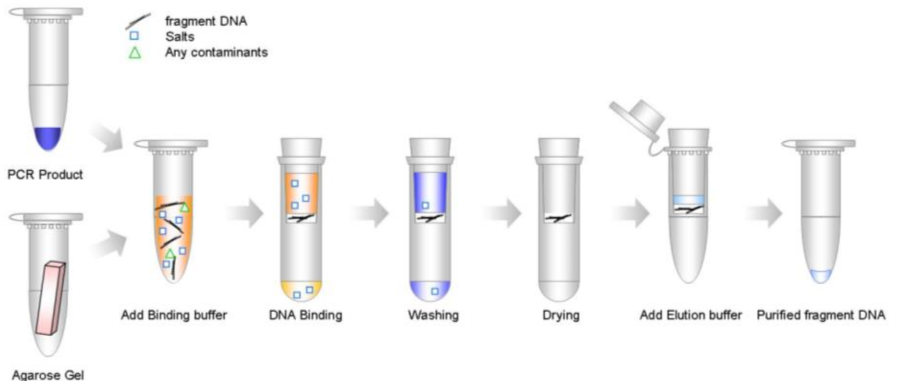
- Absolute isopropanol
- 1.5ml tube (for elution)
- Standard table-top microcentrifuge capable of a 13,000 x g centrifugal force (with rotor for 2 ml tubes)
- Incubator or thermal block
- Vortex mixer

3. Before you begin

Before you proceed, check the followings.

- Add adequate amount of isopropanol to PB Buffer. Please note the instruction on label of PB Buffer.
- Before starting extraction process, heat the EA buffer at 56 – 60°C.

4. Procedure



5. Experimental Protocol for PCR Purification

1) Add 5 volumes of PB Buffer to the PCR product, then add 1 volume of absolute isopropanol.
   If the PCR product is 20 μl, add 100 μl of PB Buffer then 20 μl of absolute isopropanol. Mix them completely by vortexing. It is not necessary to remove mineral oil.

2) Add 100 μl of BST Solution to the binding column tube (fit in a collection tube) and centrifuge for 30 sec at 13,000 rpm.

3) Discard the solution from the collection tube and reuse the collection tube.

4) Transfer the mixture to the binding column and centrifuge the column at 14,000 rpm for 1 min. We recommend to perform all centrifugation step at room temperature.

5) Pour off the flow-through and re-assemble the binding column with the 2 ml collection tube.

6) Add 500 μl of W2 Buffer to the binding column and centrifuge at 14,000 rpm for 1 min.

7) Pour off the flow-through and re-assemble the binding column with the collection tube.

8) Repeat step 6 and 7.

9) Centrifuge once more at 14,000 rpm for 1 min to completely remove residual washing buffer, and check that there is no droplet at the bottom of the binding column. Then, transfer the binding column to the new 1.5 ml microcentrifuge tube (not provided).

10) Add 30 μl of EA Buffer to the center of the binding column and wait for at least 1 min at room temperature for elution.
    If DNA fragments are larger than 3 kb, increase incubation time for 10 min at 60°C. In case of pure water, eluted fragment DNA may be denatured and unstable. EA Buffer as well as TE buffer (pH 8.0) are suitable for ordinary downstream applications such as such as sequencing, restriction enzyme digestion and ligation. On the contrary elution of fragment DNA with pure water may give the DNA denaturation and/or instability. TE buffer (pH 8.0) can be used as EA buffer except that

EDTA may interrupt the subsequent enzymatic reactions.

11) **Elute the fragment DNA by centrifugation at 14,000 rpm for 1 min.**

If more DNA yield is required, elute the sample twice and use after concentration.

6. Experimental Protocol for Gel Purification

1) **Excise the piece of agarose gel which contains desired fragment DNA as small as possible. Weigh the gel slice in a clean 1.5 ml microcentrifuge tube.**

The size of gel slice should be less than 400 mg. For gel slice >400 mg, use more than one binding column.

2) **Add 3 volumes of FB Buffer to 1 volume of the gel slice.**

If weight of the gel slice is 200 mg, add 600 µl of FB Buffer.

3) **Incubate the tube with gel slice at 50 ℃ for 10 min. Vortex the tube every 2–3 minutes during the incubation for complete dissolution.**

Complete dissolution is very important. If the gel slice doesn't melt completely, increase the incubation time.

4) **After dissolution of the gel slice, check the color of the mixture is yellow.**

If the color of the mixture is orange or red, add 10 µl of 3M sodium acetate (pH 5.0) and mix so that the color should be turned into yellow.

IMPORTANT: Color of the mixture indicates pH of the mixture. Under pH ≤ 7.5 (yellow color) the fragment DNA can effectively bind to the binding column.

5) **Add 1 volume of absolute isopropanol to 1 volume of the gel slice and mix gently.**

If weight of the gel slice is 200 mg, add 200 µl of isopropanol.

6) **Add 100 µl of BST Solution to the binding column tube (fit in a collection tube) and centrifuge for 30 sec at 13,000 rpm.**

7) Discard the solution from the collection tube and reuse the collection tube.

8) Transfer the mixture to the binding column then centrifuge the column at 14,000 rpm for 1 min.

9) Pour off the flow-through and re-assemble the binding column with the 2 ml collection tube.

10) Add 500 µl of W2 Buffer to the binding column tube and centrifuge at 14,000 rpm for 1 min.

11) Pour off the flow-through and re-assemble the binding column with the 2 ml collection tube.

12) Repeat step 10 and 11.

13) Centrifuge once more at 14,000 rpm for 1 min to completely remove residual washing buffer, and check that there is no droplet at the bottom of the binding column. Then, transfer the binding column to the new 1.5 ml microcentrifuge tube (not provided).

14) Add 30 µl of EA Buffer to the center of the binding column and wait for at least 1 min at room temperature for elution.
    If DNA fragments are larger than 3 kb, increase incubation time for 10 min at 60°C. In case of pure water, eluted fragment DNA may be denatured and unstable. EA Buffer as well as TE buffer (pH 8.0) are suitable for ordinary downstream applications such as such as sequencing, restriction enzyme digestion and ligation. On the contrary elution of fragment DNA with pure water may give the DNA denaturation and/or instability. TE buffer (pH 8.0) can be used as EA buffer except that EDTA may interrupt the subsequent enzymatic reactions.

15) Elute the fragment DNA by centrifugation at 14,000 rpm for 1 min.
    If more DNA yield is required, elute the sample twice and use after concentration.

7. Troubleshooting

1) Low yield

- Incomplete dissolution of the gel slices gives lower yield. Use the right amount of gel slices, vortex frequently during incubation, and/or increase the incubation time.
- Inadequate concentration of chaotropic salts affects DNA binding to binding column and dissolution of the gel slice. Use the right volume of FB buffer.
- Incorrect pH in binding reduces the yield. Orange or red color in FB Buffer indicates that pH value is out of range for adequate DNA binding reaction. Adjust pH of binding solution with a few microliters of 3M sodium acetate solution appropriately.
- Incorrect elution buffer with any salt may reduce the yield. Use EA Buffer for elution.

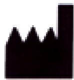
2) Eluate floats upon loading in agarose gel

Eluate may contain residual ethanol. Remaining ethanol in the sample causes floating. You must centrifuge triply and make sure that no droplet is hanging from the tip of the column. If the problem persists, let the column dry in the air for about 10 min after second centrifugation.

3) Subsequent enzymatic reaction does not work well

- High salt concentration of the eluate prevents enzyme from working. In this case, let the binding column stand for 5 min after adding W2 Buffer, then centrifuge.
- Sample contains residual W2 Buffer. Remaining ethanol interrupt the enzymatic reaction. The binding column tube must be dried completely. If the problem persists, let the binding column dry in the air for about 10 min after second centrifugation.

8. Explanation of symbol

	Catalog Number		Contains sufficient for (n) tests
	USE BY		Temperature Limitation
	Batch code		Caution, consult accompanying documents
	Manufacturer		Caution, Potential Biohazard
	DO NOT REUSE		Consult Instruction For Use

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