ISO 9001
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User's Guide

Global Genomics Partner

AccuPrep® PCR Purification Kit

Cat. No.: K-3034

Cat. No.: K-3034-1

BIONEER
bioneer corporation

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® is trademark of Bioneer Corporation.

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AccuPrep® PCR Purification Kit

Technical Manual

I. Description

This kit is designed for the purification of up to 10 μg of DNA fragment from PCR and other enzymatic products, within 5 minutes. The size range for effective purification is 100 bp - 10 kb, thus common 20 – 40mer oligonucleotides are removed. The recovery yield exceeds 80 %. Elution volume can be as little as 30 μl when concentrated product is needed. Removal of mineral oil is not necessary.

The principle of this kit is based on the adsorption of DNA in glass filter. Chaotropic salt enhances the binding of DNA onto a glass filter that is fixed in a Binding column tube. Adsorption of DNA is so selective that other proteins and salts are not adsorbed and pass through the glass filter. Washing eliminates salts and other proteins. High-purity DNA fragments are eluted with provided EL Buffer (10 mM Tris-Cl, pH 8.5) or distilled water.

Purified DNA fragment can be applied to subcloning, sequencing and other molecular biological applications.

II. Kit Components

The product has been designed to perform 50 or 200 purifications, and will maintain performance for at least a year.

***AccuPrep*® PCR Purification Kit**

Cat. No.	K-3034	K-3034-1
Reagents	**200 kit**	**50 kit**
PCR Binding Buffer (PB) Store at room temperature. Handle carefully! This buffer contains irritant chaotropic reagent.	**120 ml**	**30 ml**
Washing Buffer (WB) Add absolute ethanol to each bottle before use. Store at room temperature.	**2 X 25 ml**	**12 ml**
Elution Buffer (EL) (10 mM Tris-Cl, pH 8.5) Store at room temperature.	**15 ml**	**5 ml**
Columns and tubes		
Binding column tubes	200 ea	50 ea
2 mL tubes for filtration	200 ea	50 ea
1.5 mL tubes for elution	200 ea	50 ea

- Additional materials required

1. Absolute ethanol
2. Absolute isopropanol
3. Microcentrifuge tube (1.5 ml)
4. Table-top microcentrifuge 10,000 x g (13,000 rpm)
5. Incubator or thermal block

III. Before You Begin

Before you proceed, check if you have added the specified volume of absolute ethanol (100 ml for K-3034, 48 ml for K-3034-1) to WB Buffer. speed of all the centrifuge steps is set at 10,000 x g (13,000 rpm) in a table-top microcentrifuge.

IV. Experimental Protocol

1. **Add 5 volumes of PB Buffer to 1 volume of the PCR reaction.**
   If the PCR product is 20 μl, add 100 μl of PB Buffer. Mix them completely. It is not necessary to remove mineral oil.

2. **Place a Binding column tube in a 2 ml tube.**

3. **Apply the sample to the Binding column tube to bind DNA.**

4. **Centrifuge for 30-60 sec to make the sample pass through the Binding column tube.**

5. **Discard flow-through and place the Binding column tube in the same tube.**

6. **Add 500 μl of WB Buffer to the Binding column tube and centrifuge for 30-60 sec to wash.**
   This step removes salts and soluble impurities in the Binding column tube. The loss of DNA in this step is negligible.

7. **Discard flow-through and place the Binding column tube in the same tube again.**

8. **Repeat the washing procedure using 500 μl of WB Buffer.**

9. **Centrifuge the Binding column tube for an additional 2 min for drying.**
   Remove WB Buffer completely, and make sure there is no droplet hanging from the tip of the column. Residual WB Buffer may interrupt the subsequent enzymatic reactions.

10. **Place the Binding column tube in a clean 1.5 ml tube.**

11. **Add 30 μl of EL Buffer to the center of the Binding column filter, and let the column stand for 1 min.**
    It is critical that the EL Buffer is completely absorbed in the glass filter. The volume can be adjusted from 30 μl to 50 μl. The more the volume of the elution buffer, the higher the recovery. It assures more than 80% recovery with 30 μl of EL Buffer. If DNA fragments are larger than 3 kb, increase incubation time to 10 min and temperature at 60°C.
    Maximum yield is achieved at pH 7.0-8.5 with the EL Buffer. In case of pure water, eluted DNA may be denatured and unstable. Provided EL Buffer satisfies these requirements and does not cause any problems in ordinary enzymatic reactions such as sequencing, restriction enzyme digestion, and ligation. Common TE Buffer (pH 8.0) does also give satisfactory result.

    *Caution: EDTA may interrupt the subsequent enzymatic reactions.

12. **Centrifuge for 1 min to elute.**
    If you want more quantity, repeat step 11 and 12 with 30 μl of additional EL Buffer.

V. Problem Solving

1. Low yield

1) Did you mix PB Buffer and PCR products completely? Inadequate concentration of chaotropic salts dose not enhance the adsorption of DNA to the Binding column tube.

2) Did you add adequate amount of ethanol to the WB Buffer? Concentrated WB Buffer may wash away the adsorbed DNA.

3) Incorrect elution buffer may reduce the yield. Elution buffer should not contain too much salt. The pH of the EL Buffer should be adjusted to 7.0-8.5.

2. Sample floats upon loading in agarose gel

Sample may contain residual WB Buffer, which causes floating of sample. You must centrifuge 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

1) High salt concentration of the sample prevents enzyme from working. In this case, let the Binding column tube stand for 5 min after adding WB Buffer, then centrifuge.

2) Sample contains residual WB Buffer. Remaining ethanol interrupt enzymatic reactions. The Binding column tube

must be dried completely. If the problem persists, let the Binding column tube dry in the air for about 10 min after second centrifugation.

3) Co-eluted glass fibers interfere with enzyme. Centrifuge for 1 min and transfer the supernatant to a new tube.

VI. Supplement

1. Recovery yield

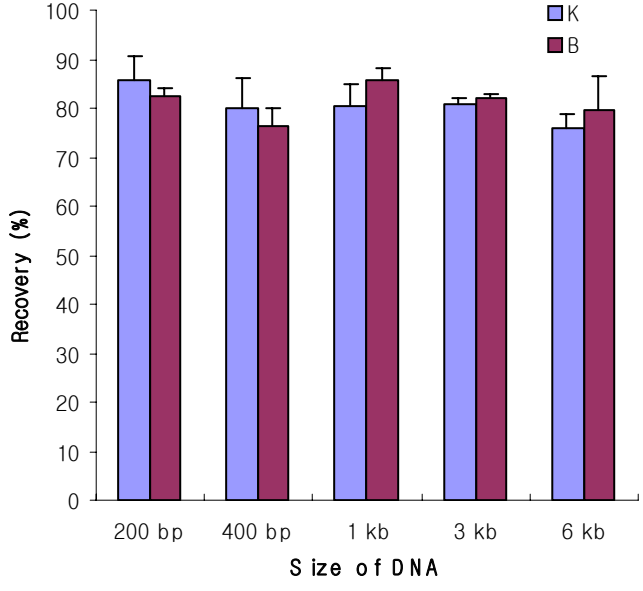

Figure 1. Recovery percentage after purification of DNA amplified by PCR. 75% - 85% of the DNA was recovered regardless of DNA size (0.2 – 6.0 kb).
B: Bioneer's *AccuPrep*® PCR Purification Kit
K: Competitor's PCR Purification Kit

2. Quality of DNA after purification

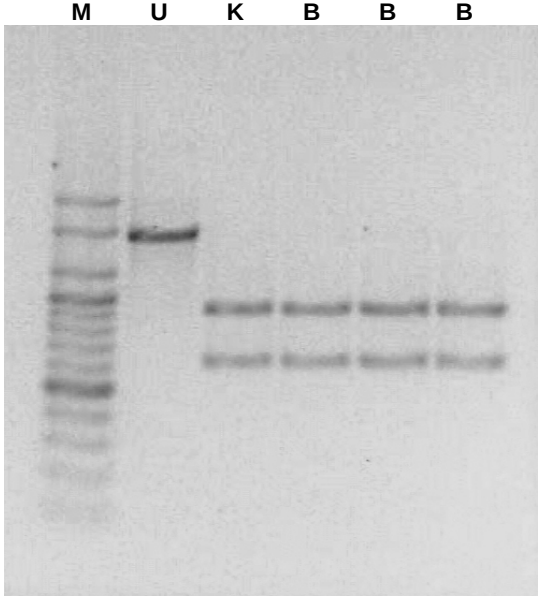

Figure 2. 1.5 kb DNA amplified by PCR was purified, digested with *Hind* III and separated by 1.2% agarose gel electrophoresis. DNA was digested completely in 1.5 hr in standard condition.
B: Bioneer's *AccuPrep*® PCR Purification Kit
K: Competitor's Kit
M: 100 bp ladder
U: uncut (1.5 kb)

3. Sequencing quality of purified PCR product

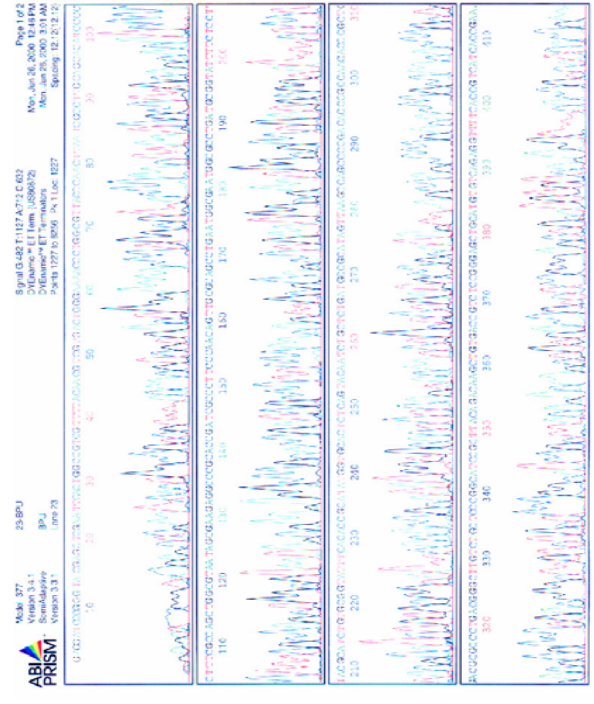

Figure 3. The sequence was analyzed by ABIPRISM™ #377 with DYEnamic™ ET Terminators (Perkin-Elmer Corp.). Up to 600 bases of readable sequence are routinely obtained.

4. The removal of primer dimers after purification

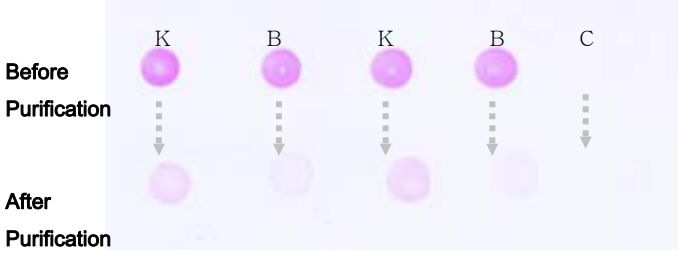

Figure 4. The removal of the remaining primer dimers (25 bp) after purification.
B: Bioneer's *AccuPrep*® PCR Purification Kit
K: Competitor's product
C: negative control (water).

Primer dimers were labeled with fluorescein. Dot blot membrane was scanned by AXON's GenePix 4000A.

VII. 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**, 635-644

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