ISO 9001
Certified

User's Guide

Global Genomics Partner

AccuPrep® Gel Purification Kit

Cat. No.: K-3035

Cat. No.: K-3035-1

BIONEER
bioneer corporation

Safety Warnings and Precautions

For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Wear gloves when you treat irritant or harmful reagents.

Warranty and Liability

All BIONEER products undergo extensive Quality Control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. Any liability incurred by BIONEER to the customer is limited to the replacement of the products. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days, and returning the product to BIONEER for examination.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system, from product development and production to quality assurance and supplier qualification, meets 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 producs.

Trademarks

AccuPrep® is trademark of Bioneer Corporation.

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AccuPrep® Gel Purification Kit
Technical Manual

I. Description

This kit is designed for the purification of up to 10 μg DNA fragment from low-melting, TAE and TBE agarose gel within 15 minutes. The size range for effective purification is about 70 bp – 10 kb. The recovery yield exceeds 70%. 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 glass filter. Chaotropic salts enhance not only melting of agarose gel but binding of DNA onto the glass filter that is fixed in a binding column tube. Adsorption of DNA is so selective that molten agarose and salts are not adsorbed and pass through the binding column tube. Washing eliminates salts and residual agarose gel. 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 for 50 or 200 purifications, and will retain performance for at least one year.

***AccuPrep*® Gel Purification Kit**

Cat. No.	K-3035	K-3035-1
Reagents	**200 kit**	**50 kit**
Gel Binding Buffer (GB) Store at room temperature. Handle carefully! This Buffer contains chaotropic reagents that is irritant.	**4 X 100 ml**	**100 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 the following requirements are met.

1. Absolute isopropanol and heating block (or water bath) at 60°C are required.

2. 3M Sodium Acetate Buffer (pH 5.0) may be needed.

3. Did you add the specified volume of absolute ethanol to WB Buffer? If not, add absolute ethanol (100 ml for K-3035, 48 ml for K-3035-1) before use.

* The 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. **Excise the DNA band of agarose gel as small as possible and 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 tube.

2. **Add 5 volumes of GB Buffer to 1 volume of the gel slice for the DNA fragment of 100 bp – 2 kb. Otherwise, refer the table below.**
   For example, if the weight of the gel is 150 mg that contains 1 kb fragment, add 750 μl of GB Buffer.

3. **Incubate at 60°C for 10 min. After incubation, ensure that there are no unwanted particles of agarose gel.**
   Vortexing the tube every 2-3 min enhances the melting

efficiency. If the gel is not melted, increase incubation time. Residual agarose gel may inhibit subsequent enzymatic reaction.

4. **Check if the color of the mixture is yellow.**

DNA < 100 bp and > 2% agarose gel	10 volumes of GB Buffer
100 bp ≤ DNA < 2 kb	5 volumes of GB Buffer
DNA ≥ 2 kb	3 volumes of GB Buffer

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

5. **Add 1 gel slice volume of absolute isopropanol and mix gently.**
If the initial weight of the gel is 150 mg, add 150 μl of isopropanol. Do not centrifuge without mixing. This step increases the yield of DNA fragments < 200 bp and > 3 kb. For DNA fragments between 200 bp and 3 kb, addition of isopropanol has no effect on yield.

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

7. **Apply the sample to the Binding column tube.**
Maximum 750 μl of the mixture can be loaded. Load and spin twice if it is more than 750 μl.

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

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

10. **(Optional) Add 500 μl of GB Buffer to the Binding column tube and centrifuge for 1 min.**
This step removes any invisible, remaining agarose gel fragment. It is recommended for preparing DNA for sequencing.

11. **(Optional) Discard flow-through and place the Binding column tube in the same tube.**

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

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

14. **Repeat the wash procedure using 500 μl of WB Buffer.**

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

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

17. **Add 30 μl of EL Buffer to the center of the Binding column filter as possible, and let the column stand for 1 min.**

It is critical that the Buffer is completely absorbed into 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 70% recovery with 30 μl.

If DNA fragments are larger than 3 kb, increase incubation time to 10 min and temperature to 60°C.

Maximum yield is achieved with the Buffer at pH 7.0-8.5. 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 inhibit subsequent enzymatic reactions.

18. Centrifuge for 1 min to elute.

If you want more quantity, repeat step 17 and 18 with additional 30 μl of EL Buffer.

V. Problem Solving

1. Low yield

1) Incomplete melting lowers the yield. Not only inadequate concentration of chaotropic salts affect the adsorption but also DNA encased in agarose gel does not bind to the glass filter.

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

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

4) Incorrect binding conditions like high pH reduces the yield. GB Buffer contains pH indicator which color is yellow but it turns to red or orange when the pH is out of range. In this case, several drops of sodium acetate Buffer adjust the pH of the solution appropriately.

5) Too much gel slice is loaded. More than 400 mg agarose gel is loaded, and then binding efficiency of DNA may be lowered.

2. Sample floats upon loading in agarose gel

Sample may contain residual WB Buffer. 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

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 into the column, then centrifuge.

2) Sample contains residual WB Buffer. Remaining ethanol inhibit 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 into a new tube.

VI. Supplement

1. Recovery yield

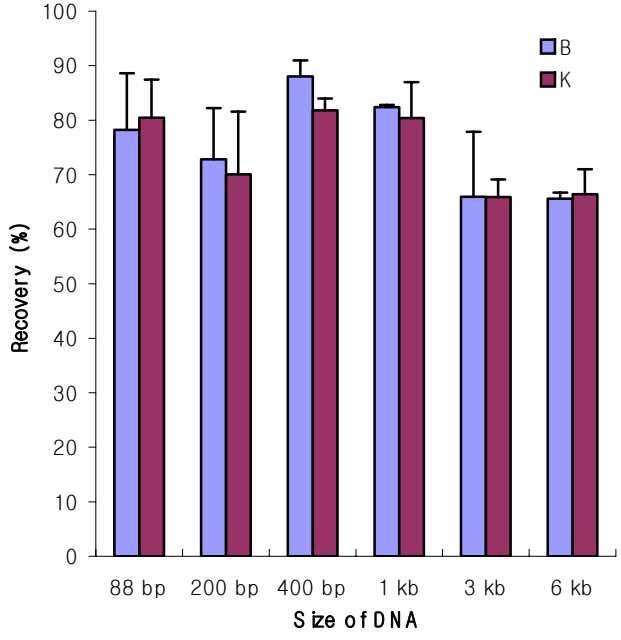

Figure 1. Recovery percentage after purification of DNA from agarose gel. 65% - 75% of the DNA were recovered regardless of DNA size (0.2 - 6.0 kb).
B: Bioneer's *AccuPrep*® Gel Purification Kit
K: Competitor's Gel Purification Kit

2. Quality of DNA after purification

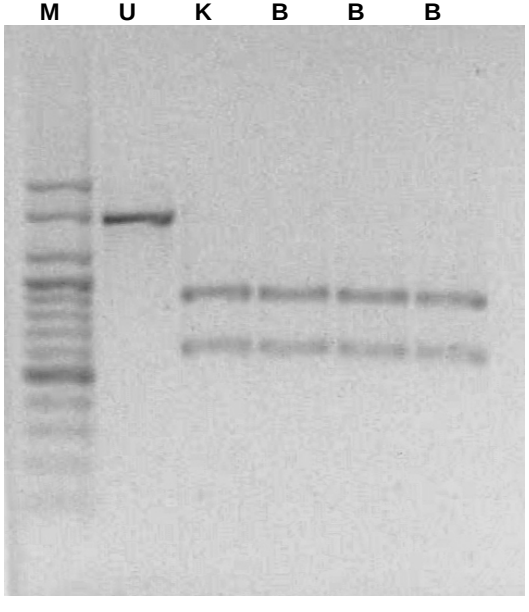

Figure 2. 1.5 kb DNA was purified from agarose gel. It was digested with *Hind* III and separated by 1.2% agarose gel electrophoresis. DNA was digested completely in 1.5 hr in standard condition.
B: Bioneer's *AccuPrep*® Gel Purification Kit
K: Competitor's kit
M: 100 bp ladder
U: uncut (1.5 kb)

3. Ligation test of gel-purified DNA

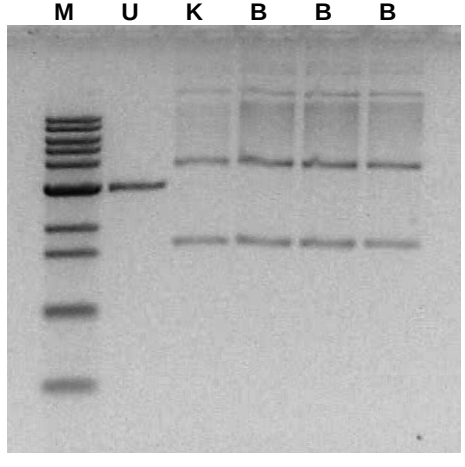

Figure 3. Re-ligation of gel-purified DNA. Gel-purified DNA was ligated by T4 DNA ligase after purification using Bioneer's *AccuPrep*® Gel Purification Kit (B) and competitor's kit (K).
M: 1 kb ladder
U : negative control (no ligation)
A plasmid DNA (pBluescript II, 2.96 kb, Stratagene®) was digested with *Hind* III and gel-purified.

4. Sequencing quality of gel-purified DNA

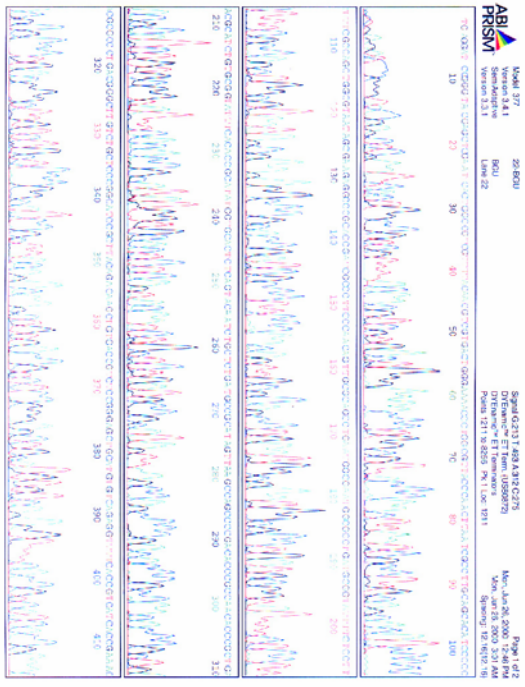

Figure 4. The sequence was analyzed by ABIPRISM™ #377 with DYEnamic™ ET Terminators (Perkin-Elmer Corp.). Up to 600 base of readable sequence is routinely obtained.

VII. Reference

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