User's Guide

# *AccuPrep*® PCR Purification Kit
# *AccuPrep*® Gel Purification Kit

REF

K-3034
K-3034-1
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BIONEER
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# *AccuPrep*® PCR Purification Kit

# *AccuPrep*® Gel Purification Kit

# User's Guide

**Version No.: 2.2 (2016-04)**

**Please read all the information in booklet before using the unit**

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## Safety Warnings and Precautions

*AccuPrep*® PCR & Gel Purification Kit is developed and sold for research purposes only. It is not recommended for human or animal diagnostic use, unless cleared for such purposes by the appropriate regulatory authorities in the country of use.

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 Sheets (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 Bioneer's quality management system from product development to production to quality assurance and supplier qualification meets or exceeds the world-class quality standards.

## Trademarks

*AccuPrep*® is trademark of Bioneer Corporation in Korea.

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## I. Description

*AccuPrep*® PCR Purification Kit is designed for the purification of up to 10 μg of PCR product from PCR mixture and other enzymatic products, within 5 minutes. And also *AccuPrep*® Gel Purification kit is designed for purification of up to 10 μg of fragment DNA from low-melting, TAE and TBE agarose gel within 15 minutes. The size range for effective purification is about 70 bp –10 kb. The average recovery yield exceeds 70% ~ 90%. 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. And chaotropic salts enhance melting of agarose gel but binding of DNA onto the silica based membrane that is packed 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 Elution Buffer or distilled water. Purified DNA fragment can be applied to subcloning, sequencing and other molecular biological applications.

## II. Kit Components

| | PCR Purification Kit | |
|---|---|---|
| Cat. No | K-3034 | K-3034-1 |
| Buffer ① (PCR Binding Buffer) | 120 ml | 25 ml |
| Buffer ② (Washing Buffer) | 25 ml × 2 ea | 6 ml × 2 ea |
| Buffer ③ (Elution buffer) | 15 ml | 15 ml |
| DNA binding column tube | 200 ea | 50 ea |
| User's Guide | 1 ea | 1 ea |
| One Page Protocol | 1 ea | 1 ea |

| | Gel Purification Kit | |
|---|---|---|
| Cat. No | K-3035 | K-3035-1 |
| Buffer ① (Gel Binding Buffer) | 120 ml × 2 ea | 30 ml × 2 ea |
| Buffer ② (Washing Buffer) | 25 ml × 2 ea | 6 ml × 2 ea |
| Buffer ③ (Elution Buffer) | 15 ml | 15 ml |
| DNA binding column tube | 200 ea | 50 ea |
| User's Guide | 1 ea | 1 ea |
| One Page Protocol | 1 ea | 1 ea |

※ Buffer ① contains chaotropic salt and should be handled with care. Chaotrophic salts can makes highly reactive compounds when mixed with disinfecting agent such as bleach.

※ All buffer and DNA binding columns can be stored under room temp.

## III. Before You Begin

Before you start your prep, please check the followings.

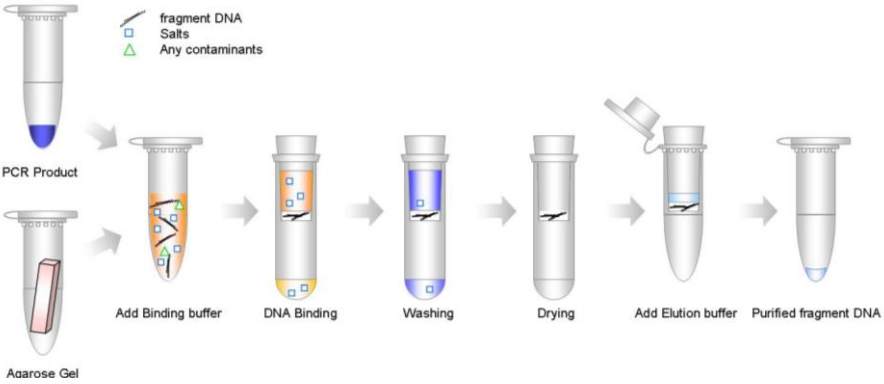
1. Chemicals
- Did you add absolute EtOH to Buffer ② as described below?

| Cat. No. | K-3034 | K-3035 | K-3034-1 | K-3035-1 |
|---|---|---|---|---|
| Buffer ② | 25 ml | | 6 ml | |
| Absolute EtOH | 100 ml | | 24 ml | |
| Total | 125 ml | | 30 ml | |

2. Equipments
- High speed refrigerated centrifuge (capable of ≥12,000 rpm, 4℃)
- Heating block (for Gel Purification Kit)

## IV. Procedure

- *AccuPrep*® PCR & Gel Purification Kit



## V. Experimental Protocol for PCR Purification

1. Add 5 volumes of Buffer ① (PCR Binding Buffer) to the PCR product.
   If the PCR product is 20 μl, add 100 μl of Buffer ①. Mix them completely by vortex. It is not necessary to remove mineral oil.

2. Transfer the mixture to the DNA binding column tube and centrifuge for 1 min. at 13,000 rpm or 10000g.

3. Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0ml collection tube.

4. Add 500 μl of Buffer ② to the DNA binding column tube and centrifuge for 1 min. at 13,000 rpm or 10000g.
   This step removes salts and soluble impurities in the DNA binding column tube. The loss of DNA in this step is negligible.

5. Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0ml collection tube.

6. Repeat Step 4. and 5.

7. Dry by additional centrifugation at 13,000 rpm or 10000g for 1 min. to remove the residual ethanol and transfer the DNA binding filter column to the new 1.5 ml micro-centrifuge tube (not provided).

8. Add 30 μl of Buffer ③ to the center of the DNA binding filter column, and wait for at least 1 min. at room temp. for elution.
   If DNA fragments are larger than 3.0 kb, increase incubation time for 10 min. and temperature at 60°C. In case of pure water, eluted fragment DNA may be denatured and unstable. Provided Buffer ③ 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.

9. Elute the fragment DNA by centrifugation at 13,000 rpm or 10000g for 1 min.
   If you want more quantity, elute the sample twice and use after concentrating process.

## VI. Experimental Protocol for Gel Purification

1. **Excise the fragment DNA from the agarose gel, as small as possible and weigh the gel slice in a clean 1.5 ml micro-centrifuge 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 3 volumes of Buffer ① (Gel Binding Buffer) to 1 volume of the gel slice.**
   If the weight of the gel slice is 200 mg, add 600 µl of Buffer ①.

3. **Incubate at 60 ℃ for 10 min. and vortex the tube every 2-3 min. during incubation for the complete dissolving.**
   Important!!! Complete dissolving is very important. If the gel slice doesn't melting, increase the incubation time.

4. **After dissolving 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. The color should be turned into yellow.
   Important!!! The color of the mixture is indicated pH of the mixture which related with DNA binding. pH ≤ 7.5 (yellow color), the fragment DNA can effectively bind to the DNA binding filter.

5. **(Optional) Add 1 volume of absolute isopropanol to 1 volume of the gel slice, and mix gently.**
   If the weight of the gel slice is 200 mg, add 200 µl of isopropanol. This step will increase the typical yield, DNA size <200 bp and >3 kb. For DNA fragments between 200 bp and 3 kb, addition of isopropanol has no effect on yield.

6. **Transfer the mixture to the DNA binding column tube and centrifuge for 1 min. at 13,000 rpm or 10000g.**

7. **Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0 ml collection tube.**

8. **Add 500 µl of Buffer ② to the DNA binding column tube and centrifuge for 1 min. at 13,000 rpm or 10000g.**
   This step removes salts and soluble impurities in the DNA binding column tube. The loss of DNA in this step is negligible.

9. **Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0 ml collection tube.**

10. **Repeat Step 8. and 9.**

11. **Dry by additional centrifugation at 13,000 rpm or 10000g for 1 min. to remove the residual ethanol and transfer the DNA binding filter column to the new 1.5 ml micro-centrifuge tube (not provided).**

12. **Add 30 µl of Buffer ③ to the center of the DNA binding filter column, and wait for at least 1 min. at room temp. for elution.**
    If DNA fragments are larger than 3.0 kb, increase incubation time for 10 min. and temperature at 60°C. In case of pure water, eluted fragment DNA may be denatured and unstable. Provided Buffer ③ 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.

13. **Elute the fragment DNA by centrifugation at 13,000 rpm or 10000g for 1 min.**
    If you want more quantity, elute the sample twice and use after concentrating process.

## VII. Troubleshooting

### 1. Low yield

1) Incomplete dissolving of the gel slices gives lower yield. Inadequate concentration of chaotropic salts affect the DNA binding to DNA binding filter and dissolving the gel slice.
2) Incorrect binding conditions like high pH reduced the yield. Buffer ① 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.
3) Did you add adequate amount of ethanol to the Buffer ②? Concentrated Buffer ② may wash away the adsorbed DNA.
4) Incorrect elution buffer may reduce the yield. Buffer ③ should not contain any salts.

### 2. Sample floats upon loading in agarose gel

Sample 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

1) High salt concentration of the sample prevents enzyme from working. In this case, let the DNA binding column tube stand for 5 min after adding Buffer ②, then centrifuge.
2) Sample contains residual Buffer ②. Remaining ethanol interrupt the enzymatic reaction. The DNA binding column tube must be dried completely. If the problem persists, let the DNA binding column tube dry in the air for about 10 min after second centrifugation.

## VIII. References

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. Taylor, R. G., Walker, D. C. and McInnes, R. R. (1993) Nucleic Acids Res., 21, 1677-1678

4. Melzak, K. A. et al. (1996), J Colloid and Interface Sci., 181, 635-644

5. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning 2nd Ed.

## IX. Explanation of symbols

| | | | | | |
|---|---|---|---|---|---|
|  | Catalog Number |  | Contains sufficient for (n) tests |  | USE BY |
|  | Batch code |  | Caution, consult accompanying documents |  | Temperature Limitation |
|  | Manufacturer |  | Caution, Potential Biohazard |  | DO NOT REUSE |
|  | Consult Instruction For Use |  | In Vitro Diagnostics Medical Device |  | Authorized Representative in the European Community |

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