ISO 9001
Certified

User's Guide

Global Genomics Partner

AccuPrep® Stool DNA Extraction Kit

Cat. No.: K-3036

BIONEER
bioneer corporation

V109C3

Safety Warnings and Precautions.

For research use only. It is not recommended to diagnose disease in humans or animals. DO NOT use to humans or animals.

Wear gloves when you are handling irritant or harmful reagents.

Warranty and Liability

All BIONEER products are tested under extensive Quality Control procedures. BIONEER guarantees the quality under the warranty period. Any problems should be reported immediately. Liability is conditional upon the customer providing full details of the problem to BIONEER. Once the problem occurred, customer must report 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.

QC Testing

Each lot of BIONEER's product is carefully tested in the quality control team.

Trademarks

AccuPrep® is trademark of Bioneer Corporation.

Contents

AccuPrep® Stool DNA Extraction Kit
Technical Manual

I. Introduction

The *AccuPrep*® Stool DNA Extraction Kit can quickly and conveniently extract up to 5μg of DNA from 100~200 mg of stool. In the presence of chaotropic salt, DNA is bound to glass fibers fixed in a column. Proteins and other contaminants are removed through washing stems, and the DNA isolated and eluted in the final elution step. The process does not require the use of dangerous organic solvents or ethanol precipitation steps. Also, DNA is efficiently extracted regardless of the condition of the stool.

Advantages :

1. DNA can be prepared faster and more conveniently.
2. Other cellular components besides nucleic acids, especially protein, nucleases, and other contaminants or inhibitors are completely eliminated, improving the efficiency and reproducibility of PCR.
3. As there are no precipitation or organic solvent-utilizing steps, damage to DNA is minimized.
4. The DNA prepared can be used in a variety of applications.

II. Kit Components

The product has been designed for 100 purifications, and will retain performance for at least one year.

K-3036 *AccuPrep*® stool DNA Extraction Kit

Reagents	
Proteinase K, lyophilized	25 mg X 2
One vial with 25 mg lyophilized proteinase K. Dissolve in nuclease-free water. Storage at -20℃ is recommended to prolong the activity of proteinase K, but repeated freezing and thawing should be avoided. For this reason, storage of aliquots of proteinase K is recommended.	
Stool Lysis buffer (S**L**)	50 ml
Mix SL buffer thoroughly by shaking before use. SL buffer is stable for 2 year when stored at room temperature.	
Binding buffer (**ST**)	50 ml
Mix ST buffer thoroughly by shaking before use. ST buffer is stable for 2 year when stored at room temperature.	
*** NOTE:** Do not add Proteinase K directly to Binding buffer.	
Washing buffer 1 (**W1**)	40 ml
W1 buffer is supplied as a concentrate. Before using for the first time, add a 30ml of absolute ethanol. W1 buffer is stable for 2 year when stored closed at room temperature.	
Washing buffer 2 (**W2**)	20 ml
W2 buffer is supplied as a concentrate. Before using for the first time, add a 80 ml of absolute ethanol. W2 buffer is stable for 2 year when stored closed at room temperature.	
Elution buffer (**E**)	25 ml
E buffer consisting of 10 mM Tris, pH 8.3 Store at room temperature.	

Columns and tubes	
DNA-binding column tubes	100 ea
2 ml tubes for filtration	100 ea
1.5 ml tubes for elution	100 ea

III. REQUIRED REAGENTS AND EQUIPMENT

1. Absolute ethanol (96 ~ 100%)
2. Absolute isopropyl alcohol
3. Microcentrifuge tubes (1.5 mL)
4. Table-top microcentrifuge for 1.5 mL and 2.0 mL microcentrifuge tubes (~ 13,000 x g)
5. Incubator or thermal block

IV. Before You Begin

Before you proceed, check if you have done the below.

i. Did you dissolve proteinase K in nuclease-free water?

ii. Did you add adequate amount of absolute ethanol to Washing buffer 1 (W1) and Washing buffer 2 (W2)?

iii. Before starting a extraction process warm the Elution buffer (E) to 70℃.

☞ The Binding buffer contains chaotropic salt, which is an irritant. Take appropriate laboratory safety precaution, and wear gloves when handling.

☞ If everything is prepared, you can proceed!

V. PROCEDURE

V-1 Extracting DNA from Stool

1. Prepare a 1.5 mL microcentrifuge tube and add 20 μl Proteinase K.

2. Add about 100~200 mg of the stool sample to the tube.

3. Buffer to the tube and mix by light vortexing for about 30 seconds. You must completely mix the sample for maximum lysis.

4. Incubate for 10 min at 60 ℃.

5. After 10 mins, centrifuge the tube at 12,000rpm for 5 mins, then transfer the supernatant to a new tube.

6. Add the supernatand to a new tube and add 200 μl Binding buffer.

7. Incubate again for 10 min at 60 ℃.

8. Add 100μl isopropanol, lightly vortex for about 5 seconds, then spin down for 10 seconds to down the liquid clinging to the walls and lid of the tube.

9. Fit the binding column into the 2ml collection tube. Transfer the liquid into the binding column, taking care to not let the lid get wet.

10. Carefully close lid and centrifuge for 1 min at 8,000 rpm.
If the liquid has not completely passed the column following

centrifugation, and is left on the column, then centrifuge again until the liquid has completely passed.

11. Following centrifugation, transfer the binding column to a new 2 ml collection tube.

12. Add 500μl Washing buffer 1 (W1) to the column, taking care so that the sides do not get wet; close the lid, and centrifuge for 1min at 8,000 rpm.

13. After centrifugation, transfer the binding column to a new 2 ml collection tube.

14. Add 500μl Washing buffer 2 (W2), taking care so that the sides do not get wet; close the lid, and centrifuge for 1min at 8,000 rpm.

15. Spin down once more at 13,000 rpm for 1 min to completely remove ethanol. Check that there are not any droplets hanging from the bottom of the binding column.
Residual Washing buffer 2 left in the binding column can hinder the following steps.

16. Transfer the binding column to a 1.5 ml collection tube, add 200μl Elution Buffer, and let stand for 1 min to allow the buffer to permeate the column.

    We recommend letting stand for about 5 min to increase DNA yield. You can add less Elution Buffer, such as, 50μl or 100μl, for a higher concentration of DNA, but the total yield will be reduced. You can also increase yield by heating the Elution Buffer to about 70℃ before adding to the column..

17. Elute by spinning down at 8,000 rpm for 1 min.
    About 180μl of eluate can be recovered after using 200μl Elution buffer. For maximum total yield, you can repeat the elution step.

    The eluted DNA solution can directly be used, or stored at 4℃, or –20℃ longer storage periods.

    You can obtain about 5 μg of total DNA (2.5 ng/μL) from about 100 mg of Stool when using 200 μl Elution buffer.

VI. Troubleshooting

1. A low yield or purity of DNA is obtained.

1) The kit may have been stored under non-optimal conditions. → Store kit at 15-25℃ at all times upon arrival.

2) Buffers or other reagents may have been exposed to conditions that reduced their effectiveness. → Store all buffers at 15-25°C. Close all reagent bottles tightly after each use to preserve pH, stability, and to avoid contamination. After any lyophilized reagent is constituted, aliquot it and store the aliquot at either 2 ~ 8℃or -15 to -25℃ (as directed in the instruction manual).

3) Ethanol may not have been added to the Washing Buffers. → Add absolute ethanol to all Wash Buffers before using. After adding ethanol, mix the Wash Buffer

well and store at 15 ~ 25℃. Always mark the Wash Buffer vial to indicate whether ethanol has been added or not.

4) Reagents and samples may not have been completely mixed. → Always mix the sample tube well after adding each reagent.

2. There is a low recovery of DNA following elution.

You may not have used the optimal reagent for eluting the DNA. An alkaline pH is required for optimal elution. → Next time, do not use water to elute DNA. Use the Elution Buffer included in the kit.

3. There incomplete or no restriction enzyme cleavage of DNA extracted from the kit.

Glass fibers, which can be coeluted along with the DNA, may inhibit enzyme reactions → After the final elution step has been completed, remove column filter from tube containing the eluted sample and spin the sample tube for 1 minute at maximum speed. Glass fibers may be visible at the bottom of the tube. Transfer the supernatant into a new tube, without disturbing the glass fibers at the bottom of the original tube.

4. The absorbency (A_{260}) reading of product is too high.

Glass fibers which can coelute with nucleic acid, can scatter light, resulting in a higher absorbency reading. → See the above method for removing glass fibers.

5. There is a low yield of DNA.

1) Proteinase K may not have been completely solubilized. → Take the following steps to completely solubilize the lyophilized Proteinase K: 1. Pipette 1.25ml of double distilled water into the glass vial containing lyophilized Proteinase K. 2. Close and invert the vial until all the lyophilizate is dissolved. 3. Aliquot the reconstituted enzymes, mark each aliquot, and store at –15 to -25°C. **Note :* Proteinase K reconstituted this way is stable for 12 months when stored properly.

2) The lysis may have been incomplete. → Mix sample immediately after adding Proteinase K. Always mix the lysate thoroughly with isopropanol before adding the sample to the column filter tube.

6. There is a white precipitate in Buffer SL or Buffer ST.

A white precipitate may form in Buffer SL or Buffer ST after prolonged storage at low temperature. → Any precipitate in SL Buffer or ST Buffer must be dissolved by incubating the buffer at 70°C. The precipitate has no effect on function, and dissolving the precipitate at high temperatures will not improve yield or quality of the purified nucleic acids.

VII. Appendix

1. **Typical Yields**

The yield and purity of genomic DNA is variable depending on the sample type and condition. The table below shows typical experimental results.

Sample	Amount	Total Yield (μg)
Stool	100 mg	2 ~ 5

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. Melzak, K. A. *et al*. (1996), *J Colloid and Interface Sci*., **181**, 635-644
4. Taylor,R. G., Walker, D. C. and Mclnnes, R. R. (1993) *Nucleic Acids Res*., **21**, 1577-1678
5. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning* 2nd ed.

BIONEER CORPORATION

Headquarters
49-3,Munpyeong-dong,
Daedeok-gu,Daejeon306-220,
Korea
Phone:+82-42-936-8500
Fax:+82-42-930-8600

Seoul Office
412, 1580, DMC Hi-tech industry
center, Sangam-dong, Mapo-gu,
Seoul, 121-835, Republic of Korea
Phone:+82-2-598-1094
Fax:+82-2-598-1096

USA Bioneer, Inc.
1000 Atlantic Avenue, Alameda, CA
94501 USA
Toll free : 1-877-264-4300
Fax : 1-510-865-0350
E-mail:order.usa@bioneer.com

Order
Domestic only: 1588-9788
E-mail: order@bioneer.co.kr
URL: www.bioneer.com