AccuPrep® Plant Genomic DNA Extraction Kit

User's Guide

Σ

100

Version No.: 2.0 (2018-02)

Please read all the information in booklet before using the unit

Bioneer Corporation
8-11, Munpyeongseo-ro, Daedeok-gu, Daejeon 34302, Republic of Korea
Tel: +82-42-930-8777
Fax: +82-42-930-8688
Email: sales@bioneer.co.kr
www.bioneer.co.kr
Safety Warnings and Precautions
For research use only
Not recommended for disease diagnose in 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 occurs, 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.

Trademark
AccuPrep® is a trademark of Bioneer Corporation.

Copyright
Copyright 2018. Bioneer Corporation. All Rights Reserved

Notice
Bioneer corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice. All information provided here is subject to change without notice.
CONTENTS

I. DESCRIPTION ...........................................1

II. KIT COMPONENTS .........................................2

III. BEFORE YOU BEGIN .......................................3

IV. EXPERIMENTAL PROTOCOL ............................3

V. TROUBLESHOOTING .................................5

VI. REFERENCES ................................................7

VII. EXPLANATION OF SYMBOLS .....................7
I. Description

AccuPrep® Plant Genomic DNA Extraction Kit is designed for quick and convenient extraction of DNA from 100 mg of various plants. In the presence of chaotropic salt, DNA binds to glass fibers fixed in a column. Proteins and other contaminants are removed during washing steps, and the DNA is isolated and eluted in the final elution step. The process does not require the use of organic solvents or ethanol precipitation step; therefore, it is ideal for safe and convenient extraction DNA from a variety of botanical sources such as beans and corns.

Advantages

1. DNA can be prepared more promptly and conveniently.
2. Other cellular components besides nucleic acids, such as protein, nucleases, and other contaminants or inhibitors are completely eliminated, improving the efficiency and reproducibility of PCR.
3. As no precipitation or organic solvent is used, damage to DNA is minimized.
4. Prepared DNA can be used in a variety of applications.
II. Kit components

This kit provides for 100 preparations and will maintain performance for at least two years under standard storage conditions.

**AccuPrep® Plant Genomic DNA Extraction Kit (K-3031)**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Qty.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteinase K, lyophilized</strong></td>
<td>25 mg x 2</td>
</tr>
<tr>
<td>One vial includes 25 mg lyophilized Proteinase K. Dissolve one vial in 1,250 μl of nuclease–free water. Dissolved Proteinase K is stable when stored at 4°C. Storage at −20°C is recommended to prolong the activity, but repeated freezing and thawing should be avoided.</td>
<td></td>
</tr>
<tr>
<td><strong>RNase A, lyophilized</strong></td>
<td>24 mg x 2</td>
</tr>
<tr>
<td>One vial includes 24 mg lyophilized RNase A. Dissolve one vial in 600 μl of nuclease–free water. Dissolved RNase A is stable when stored at 4°C. Storage at −20°C is recommended to prolong the activity, but repeated freezing and thawing should be avoided.</td>
<td></td>
</tr>
<tr>
<td><strong>PL Buffer</strong></td>
<td>40 ml</td>
</tr>
<tr>
<td>Mix PL buffer thoroughly by shaking before use. PL buffer is stable for 2 years when stored at room temperature.</td>
<td></td>
</tr>
<tr>
<td><strong>PC Buffer</strong></td>
<td>25 ml</td>
</tr>
<tr>
<td>Mix PC buffer thoroughly by shaking before use. PC buffer is stable for 2 years when stored at room temperature.</td>
<td></td>
</tr>
<tr>
<td><strong>WA1 Buffer</strong></td>
<td>60 ml</td>
</tr>
<tr>
<td>WA1 buffer is supplied as a concentrate. Before using for the first time, add absolute ethanol. WA1 buffer is stable for 2 years when stored closed at room temperature.</td>
<td></td>
</tr>
<tr>
<td><strong>W2 Buffer</strong></td>
<td>80 ml</td>
</tr>
<tr>
<td>W2 buffer is stable for 2 years when stored closed at room temperature.</td>
<td></td>
</tr>
<tr>
<td><strong>EA Buffer</strong></td>
<td>25 ml</td>
</tr>
<tr>
<td>10 mM Tris–HCl (pH8.5). Store at room temperature.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Columns and tubes</th>
<th>Qty.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Binding column</strong></td>
<td>100 ea</td>
</tr>
<tr>
<td><strong>Collection tubes (for filtration)</strong></td>
<td>100 ea</td>
</tr>
<tr>
<td><strong>1.5 ml tubes (for elution)</strong></td>
<td>100 ea</td>
</tr>
</tbody>
</table>

**Additional required materials**

1. Absolute ethanol
2. Table–top microcentrifuge, 10,000 x g (13,000 rpm)
3. Incubator, thermal block, or water bath (shaking)
4. Vortex mixer
5. 1.5 ml or 2 ml tube
III. Before you begin
Before you proceed, check the followings.
1. Did you dissolve Proteinase K in 1,250 μl of nuclease-free water? Dissolved Proteinase K should be stored at 4°C.
2. Did you dissolve RNase A in 600 μl of nuclease-free water? Dissolved RNase A should be stored at 4°C.
3. Did you add adequate amount of absolute ethanol to WA1 Buffer?
4. Before starting an extraction process, warm the EA Buffer to 60°C

   WA1 Buffer contains irritant chaotropic salt. Take appropriate laboratory safety precaution, and wear gloves when handling.

IV. Experimental protocol

1) Grind ~100 mg of plant to fine powder. Put the powder and 300 μl of PL Buffer in a 1.5 ml or 2 ml tube. Powdered plants are efficient for lysis.

2) Add 20 μl of Proteinase K and 10 μl of RNase A. Mix thoroughly and make sure that you completely resuspend the sample to achieve maximum lysis efficiency.

3) Incubate the tube for 10 minutes at 60°C.

4) Add 100 μl of PC Buffer and mix thoroughly.

5) Incubate for 5 minutes on ice.

   This step precipitates detergent, proteins and polysaccharides.

6) After 5 minutes, centrifuge the tube at 13,000 rpm for 5 minutes then transfer the supernatant to a new tube.

7) Add 1.5 volumes of WA1 Buffer to the cleared lysate and mix well using a vortex mixer or by pipetting. And then spin down for 10 seconds to get the liquid clinging to the walls and lid of the tube.

8) Carefully transfer the lysate into the upper reservoir of the Binding column tube (fit in a collection tube) without wetting the rim.
9) Close the tube and centrifuge at 8,000 rpm for 1 minute.
   If the liquid has not completely passed the column following centrifugation, then centrifuge again until the liquid completely passes through.

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

11) Repeat step 8 – 10 with remaining sample.

12) Add 500 μl of WA1 Buffer to the column, without the sides getting wet; close the lid, and centrifuge for 1 minute at 8,000 rpm.

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

14) Add 500 μl of W2 Buffer, without the sides getting wet; close the lid, and centrifuge for 1 minute at 8,000 rpm.

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

16) Spin down once more at 13,000 rpm for 1 minute to completely remove ethanol. Check that there is no droplet hanging at the bottom of the binding column.
   Residual W2 Buffer left in the binding column can hinder the following steps.

17) Transfer the Binding column to a 1.5 ml tube for elution, add 50 – 200 μl of EA Buffer, and wait for 1 minute to allow the buffer to permeate the column.
   You can add less EA Buffer, for example, 50 μl or 100 μl, for a higher concentration of DNA, but the total yield will be reduced. Longer reaction time will increase the product yield. We recommend letting stand for about 5 min to increase DNA yield. You can also increase yield by heating the EA Buffer at about 60℃ before adding to the column.

18) Centrifuge at 8,000 rpm for 1 minute to elute.
   About 180 μl of eluate can be recovered after using 200 μl of EA Buffer. For maximum yield, you can repeat the elution step. The eluted DNA solution can be directly used or stored at 4℃ or −20℃ for longer storage periods.
V. Troubleshooting

1. Yield or purity of DNA is low.

   1) The kit may have been stored under non-optimal conditions.
      → Store kit at 15 – 25°C 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 use, in order to preserve pH, stability, and to avoid contamination. After constitution of any lyophilized reagent, separate into aliquots and store each aliquot at either 2 – 8°C or −25 – −15°C (as indicated in the manual).

   3) Ethanol may not have been added to the WA1 Buffer.
      → Add absolute ethanol to WA1 Buffer before using. After adding ethanol, mix the WA1 Buffer well and store at 15 – 25°C. Always mark the WA1 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 thoroughly after adding each reagent.

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

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

   You may not have used the optimal reagent for DNA elution. An alkaline pH is required for optimal elution.
   → Do not use water to elute DNA. Use the EA Buffer included in the kit.

3. There is an 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. DNA from plant samples is degraded.

There may have been nuclease activity in the unlysed tissue.
→ Tissue should be stored frozen (−20°C) after harvest until the lysis procedure starts. Use only small pieces of tissue (20–100 mg) when homogenizing the tissue sample.

5. There is a white precipitate in PL Buffer.

White precipitate may form in PL Buffer after prolonged storage at low temperature.
→ Any precipitate in PL Buffer should be dissolved by incubating the buffer at 60°C. The precipitate may not cause malfunction, however, dissolving the precipitate at high temperatures will not improve yield and quality of the purified nucleic acids.
VI. References


VII. Explanation of Symbols

<table>
<thead>
<tr>
<th>REF</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>∑</td>
<td>Contains sufficient for (n) tests</td>
</tr>
<tr>
<td></td>
<td>USE BY</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LOT</th>
<th>Batch code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>☢</td>
<td>Caution, consult accompanying documents</td>
</tr>
<tr>
<td></td>
<td>Temperature Limitation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Caution, Potential Biohazard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DO NOT REUSE</td>
</tr>
</tbody>
</table>

| Consult Instruction For Use |
Bioneeer Worldwide

Bioneeer Corporation
Address 8-11 Munpyeongseo-ro, Daedok-gu, Daejeon, 34302, Republic of Korea
Tel +82-42-930-8777 (Korea: 1588-9788)
Fax +82-42-930-8888
E-mail sales@bioneeer.com
Web www.bioneeer.com

Bioneeer Inc.
Address 1301 Marina Village PKWY, Suite 110, Alameda, CA 94501, USA
Tel +1-877-294-4300 (Toll-free)
Fax +1-510-885-0390
E-mail orders.usa@bioneeer.com
Web us.bioneeer.com

Bioneeer R&D Center
Address Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro, Bundang-gu, Seongnam-si
Gyeonggi-do, 13488, Republic of Korea
Tel +82-31-628-0600
Fax +82-31-628-0695
E-mail sales@bioneeer.co.kr
Web www.bioneeer.co.kr

Bioneeer
Innovation • Value • Discovery