User's Guide

AccuPrep®
GMO DNA Extraction Kit

REF K-3031

BIONEER
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AccuPrep® GMO DNA Extraction Kit

User's Guide

100

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Please read all the information in booklet before using the unit

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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.

QC Testing

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

Trademarks

AccuPrep®is a trademark of Bioneer Corporation.

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I. Description

The *AccuPrep*® GMO DNA Extraction Kit is designed for quick and convenient extraction of up to 5μg of DNA from 100 mg of beans or corn. 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® GMO DNA Extraction Kit (K-3031)

Reagents	
Proteinase K, lyophilized	**25 mg x 2**
One vial of 25 mg lyophilized proteinase K. Dissolve in 1.25 ml of nuclease-free water. Dissolved proteinase K is stable for up to 1 month when stored at 4℃. Storage at −20℃ is recommended to prolong the activity, but repeated freezing and thawing should be avoided.	
Lysis buffer (PL)	**50 mL**
Mix PL buffer thoroughly by shaking before use. PL buffer is stable for 2 years when stored at room temperature.	
Binding buffer (B)	**50 mL**
Mix B buffer thoroughly by shaking before use. B buffer is stable for 2 years when stored at room temperature.	
* **NOTE:** Do not add Proteinase K directly to Binding buffer.	
Washing Buffer (W1)	**40 mL**
W1 buffer is supplied as a concentrate. Before using for the first time, add 30 ml of absolute ethanol. W1 buffer is stable for 2 years when stored closed at room temperature.	
Washing Buffer (W2)	**20 mL**
W2 buffer is supplied as a concentrate. Before the first use, add 80 ml of absolute ethanol. W2 buffer is stable for 2 years when stored closed at room temperature.	
Elution Buffer (EL)	**25 mL**
10 mM Tris-HCl (pH8.5). 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

Additional required materials

1. Absolute ethanol
2. Absolute isopropanol
3. Table-top microcentrifuge, 10,000 xg (13,000 rpm)
4. Incubator, thermal block, or water bath (shaking)
5. Vortex mixer
6. 1.5 ml tube

III. Before you begin

Before you proceed, check the followings.

1. Did you dissolve proteinase K in 1.25 mL of nuclease-free water?

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

3. Before starting a extraction process warm the Elution buffer (EL) to 60℃

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

IV. Experimental protocol

Extraction Genomic DNA from Beans

1) **Grind 50 – 100 mg of beans to fine powder. Put the powder and 400 uL of Lysis Buffer (PL) in a 1.5 mL microcentrifuge tube.**
   Powdered beans are efficient for lysis.

2) **Add 20 uL of Proteinase K, mix, and incubate for 10 minutes at 60℃.**
   You should observe the formation of aggregates.

3) **After the bean powder has been lysed for 10 minutes, briefly spin down, add 400 uL of Binding buffer (B), and mix well.**

4) **Incubate for 10 minutes at 60℃.**

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

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

7) Fit the binding column into the 2ml tube and transfer the liquid into the binding column.
Be cautious not to get the lid wet.

8) Carefully close lid and centrifuge for 1 minute at 8,000 rpm.
If the liquid is left on the column, not completely passing the column following centrifugation, centrifuge again until the liquid has completely passed.

9) Following centrifugation, transfer the binding column to a new 2 mL tube.

10) Add 500 uL of washing buffer 1 (W1) to the column, without the sides getting wet; close the lid, and centrifuge for 1minute at 8,000 rpm.

11) Carefully open the binding column and pour the solution in 2 mL tube to garbage bottle.

12) Add 500 uL of Washing buffer 2 (W2), without the sides getting wet; close the lid, and centrifuge for 1 minute at 8,000 rpm.

13) 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 Washing buffer 2 (W2) left in the binding column can hinder the following steps.
Transfer the binding column to a 1.5 mL collection tube, add 200 uL of Elution Buffer, and react for 1 minute to allow the buffer to permeate the column.
Longer reaction time will increase the product yield. You can add less Elution Buffer, for example, 50 uL or 100 uL, 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.

14) Elute by spinning down at 8,000 rpm for 1 minute.
About 180 uL of eluate can be recovered after using 200 uL of Elution 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℃ 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℃. 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℃ or -15 ~ -25℃ (as indicated in the 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 thoroughly after adding each reagent.

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 Elution 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. 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 method above for removal of glass fibers.

5. There is a low yield of DNA.

1) Proteinase K may not have been completely solubilized. → Follow the steps to completely solubilize the lyophilized Proteinase K: 1. Pipette 2.5mL 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. Separate the reconstituted enzymes into aliquots, mark each one, and store them 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 low yield from tissue.

Proteinase K digestion may have been incomplete. → Be sure to slice the tissue into small pieces before the digestion and lysis steps. There are two ways to increase the incubation time: 1. Incubate tissue overnight with Proteinase K. 2. Incubate with Proteinase K for 3 ~ 4 hour, then add a fresh aliquot of Proteinase K (30 µL) and incubate for another 1 ~ 2 hr.

7. DNA from tissue 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 (20−40 mg) when homogenizing the tissue sample.

8. The final eluent from blood is still slightly colored.

You may not have washed adequately.

→ Wash the filter tube until the flowthrough is colorless.

Repeat the purification protocol by mixing 200 µL of eluent with 400 µL of Binding Buffer, then

100 µL of isopropanol.

9. There is a white precipitate in Buffer PL or Buffer B.

A white precipitate may form in Buffer PL or Buffer B after prolonged storage at low temperature.

→ Any precipitate in PL Buffer or B Buffer must be dissolved by incubating the buffer at 70°C. The precipitate causes no malfunction, however, dissolving the precipitate at high temperatures will not improve yield and quality of the purified nucleic acids.

VI. Supplementary material

1. Typical Results

The yield and purity of genomic DNA vary depending on the samples. The table below shows experimental results.

Sample	Amounts	Yield (µg)
Soybean	100mg	2 ~ 5
Maize	100mg	1 ~ 4
Potato	100mg	1 ~ 4

2. Purification yield

You can extract about 5 µg of DNA from 200 µl eluent (25 ng/µl), with an A_{260}/A_{280} ratio of 1.7 ~ 1.9 of a 100 mg of Soybean sample.

VII. References

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VII. Explanation of Symbols

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

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