USER GUIDE

AccuPrep®
Plasmid Mini Extraction Kit

Cat. No. K-3030
K-3030-1

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AccuPrep® Plasmid Mini Extraction Kit

Kit for the extraction of plasmid from bacterial cultures

User Guide

K-3030-1	K-3030
Σ 50	Σ 200

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Please read all the information in booklet before using the unit

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Intended Use

AccuPrep® Plasmid Mini Extraction Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Safety Warning and Precaution

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 Sheet (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 our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

Trademark

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Copyright

Notice

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Contents

Product Information

Components

Components	K-3030 (200 reactions)	K-3030-1 (50 reactions)	Storage
RNase A powder, lyophilized	6 mg	1.5 mg	Refer to the "Storage" below.
PA1 Buffer (Resuspension)	60 ml	15 ml	
P2 Buffer (Lysis)	60 ml	15 ml	Store at room temperature (15-25°C).
PA3 Buffer (Neutralization)	80 ml	20 ml	
PB Buffer (Endonuclease A denaturation)	75 ml	20 ml	
W2 Buffer (Washing)	80 ml x 2 ea	40 ml	
EA Buffer (Elution)	25 ml	15 ml	
BST Solution	40 ml	10 ml	
AccuPrep® Binding Column-II plus	200 ea	50 ea	
Collection Tube (Filtration)	200 ea	50 ea	
One Page Protocol	1 ea	1 ea	

Storage

The kit will maintain performance for at least two years under standard storage conditions.

All components of the kit should be stored dry at room temperature (15-25°C). PA1 Buffer is stable for 2 years when stored at room temperature. But it must be stored at 4°C after adding lyophilized RNase A. The added RNase A will retain its activity for up to 6 months. After longer periods of storage, add more RNase A powder up to 100 ng/μl.

Specifications

AccuPrep® Plasmid Mini Extraction Kit	
Starting Culture Volume	1-10 ml
Turnaround Time	< 20 minutes
Column Binding Capacity	up to 30 µg
Elution Volume	50-100 µl
Typical DNA Yield	up to 20 µg
DNA Purity	A_{260}/A_{280} > 1.8
Scale	Mini
Isolation Technology	Silica Column

*** Note:** There may be differences in measured values depending on the type of plasmids.

Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because PA3 Buffer and PB Buffer contain chaotropic salts which are irritants.
- Completely remove the protective seal in BST Solution. BST Solution may be discolored, but it does not affect nucleic acid extraction.
- Thoroughly mix PA1 Buffer by shaking before use.

Introduction

Product Description

AccuPrep® Plasmid Mini Extraction Kit is designed for extraction of highly purified plasmid DNA from cultured bacterial cells within 20 minutes. The kit is based on alkaline lysis (Birnboim *et al*., 1979) of bacterial cells. The kit employs *AccuPrep*® Binding Column-II plus with silica membrane for nucleic acid binding in the presence of chaotropic salts. This silica membrane has enough surface area to bind up to 30 μg of plasmid DNA. The kit is also available for both high-copy and low-copy plasmid DNA. The process does not require phenol/chloroform extraction, ethanol precipitation, or other time-consuming steps.

Principle

Pelleted bacterial cells are resuspended with resuspension buffer and lysed under alkaline conditions. Alkaline conditions lead to lysis, release intracellular components, and denature chromosomal DNA, plasmid DNA, and proteins. The resulting lysate is subsequently neutralized in the presence of chaotropic salts for binding of plasmid DNA onto the silica membrane in the *AccuPrep*® Binding Column-II. The high salt condition causes denatured proteins, genomic DNA, and cell debris to form insoluble aggregates, while the plasmid DNA renatures in solution. The insoluble aggregates are removed by centrifugation and cleared lysates are transferred to the silica membrane. Any salts and precipitates are eliminated by washing buffer, and highly purified plasmid DNA is eluted in a elution buffer or nuclease-free water.

Features & Benefits

- Convenient & Rapid: The process does not require phenol/chloroform extraction, ethanol precipitation, or other time-consuming steps.
- Efficient: Contaminants such as proteins and nucleases which may interfere with PCR reactions are completely removed.
- Ready-to-use: Extracted DNA is ready-to-use for various application.
- Minimized DNA damage: DNA damage is minimized by avoiding precipitation and use of organic solvents.
- High binding capacity: Uses silica-based DNA binding column with a high binding capacity of up to 30 μg.

Experimental Procedures

Before You Begin

Before proceeding, please check the following:

1. Add RNase A powder to PA1 Buffer and completely dissolve it. After adding RNase A powder, PA1 Buffer should be stored at 4°C.

2. Pre-heat EA Buffer at 60°C before use. This process is essential for maximal recovery in filter-based extraction.

3. Add volume of absolute ethanol (not provided) indicated below to PB Buffer.

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PB Buffer	75 ml	20 ml
Absolute ethanol	45 ml	12 ml
Total	120 ml	32 ml

4. g-force can be calculated as follows: rcf = 1.12 x r x $(rpm/1,000)^2$

   * **Note:** Where 'rcf' is the relative centrifugal force (in g), 'r' is the radius of the rotor in centimeters, and rpm is the speed of the centrifuge in revolutions per minute.

Plasmid Mini Extraction

- ***E. coli* cell preparation**

1. Pick a single colony from fresh cultured selective plate and inoculate the cells in the 1-5 ml of LB liquid media containing the appropriate selective antibiotics. Incubate at 37°C for 12-16 hours with shaking.

   * **Note:** Bacterial overgrowth is not recommended. DNA yields may be reduced because of cell death and inefficient lysis.
   For high-copy number plasmid DNA: 1-5ml of *E. coli* cells
   For low-copy number plasmid DNA: 1-10ml of *E. coli* cells

2. Harvest cultured cells by centrifugation at >8,000 rpm (3,000 x *g*) for 2 minutes or >3,000 rpm (600 x *g*) for 5 minutes to pellet cells. Discard the supernatant carefully with a pipette.

- **Cleared lysate preparation**

3. Resuspend the cell pellet from step 2 in 250 µl of PA1 Buffer by vortexing of pipetting.

   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

4. Add 250 µl of P2 Buffer to the sample and mix gently by inverting the tube 3-4 times.

   * **Note:** Vortexing should be avoided because this will cause shearing of genomic DNA and contamination of plasmid DNA with genomic DNA.

5. Add 350 µl of PA3 Buffer and mix immediately and thoroughly by inverting the tube 3-4 times.

   * **Note:** Be cautious not to shear genomic DNA.

6. Centrifuge the tube for 10 minutes at >13,000 rpm (16,000 x *g*) at 4°C† in a microcentrifuge.

   * **Note:** After centrifugation, white protein aggregates will appear at the bottom of the tube. If your centrifugation is not enough to get a cleared lysate, please centrifuge again.

   † It is recommended to centrifuge at 4°C to prevent degradation of cell lysate, as heat may occur during the centrifugation process.

- **Plasmid DNA Purification**

7. Add 100 µl of BST Solution to the *AccuPrep*® Binding Column-II plus fit in a Collection Tube.
8. Centrifuge at 13,000 rpm for 30 seconds and discard the flow through. Reuse the Collection Tube in step 9.

9. Apply the cleared lysate from step 6 to the *AccuPrep*® Binding Column-II plus.

10. Centrifuge at 13,000 rpm for 1 minute and discard the flow through. Reuse the Collection Tube in step 11 or 12.

11. **(Optional)** Wash the *AccuPrep*® Binding Column-II plus by adding 500 µl of PB Buffer and let stand for 5 minutes. Centrifuge at 13,000 rpm for 1 minute and discard the flow through. Reuse the Collection Tube in step 12.

    * **Note:** This step is required if you are using an *end*A+ strains, such as BL21, CJ236, HB101, JM83, JM101, JM110, LE392, NM series, PR series, Q358, PR1, TB1, TG1, T10 series, BMH71-18, and ES1301, which have high endonuclease activity. Denaturation step is not required for the DH5α, XL-Blue, BJ5183, DH1, DH20, DH21, JM103, JM105, JM106, JM107, JM108, JM109, MM294, SK1590, SK1592, SK2267, SRB, and XLO strains.

12. Wash the *AccuPrep*® Binding Column-II plus by adding 700 µl of W2 Buffer.

13. Centrifuge at 13,000 rpm for 1 minute and discard the flow through. Reuse the Collection Tube in step 14.

14. Centrifuge once more at 13,000 rpm for 1 minute to remove residual ethanol completely.

15. Place the *AccuPrep*® Binding Column-II plus in a clean 1.5 ml tube (not provided). Add 50-100 µl of EA Buffer or nuclease-free water to elute DNA and let stand for 1 minute. Centrifuge at 13,000 rpm for 1 minute.

    * **Note:** A smaller volume will result in a more concentrated solution, but total yield may be reduced. If the plasmid is low-copy or larger than 10 kb, total yield may not be sufficient. Pre-warmed EA Buffer will improve efficiency of elution (see “Before You Begin” on page 6).

16. To recover more DNA, repeat once more elution step using the eluate from step 15.

Troubleshooting

Problem	Comments
Low plasmid DNA yield or purity	• **Buffers or other reagents may have been exposed to conditions that reduce their effectiveness.** Store the reagents at room temperature (15-25°C) at all times upon arrival and keep all reagent bottles tightly closed after use to preserve pH and stability and avoid contamination. After reconstitution of the lyophilized reagents, divide it into aliquots and store small aliquots at -20°C.
	• **The cells may not have been completely resuspended with PA1 Buffer.** Resuspend completely by vortexing or pipetting.
	• **There may have been precipitated salt in P2 and PA3 Buffer.** Vortex well to re-dissolve the precipitant. If it does not re-dissolve easily, warm it at 60°C.
	• **You may not have used optimal reagents for eluting plasmid DNA.** An alkaline pH is required for optimal elution. Use EA Buffer included in the kit.
	• **PA1 Buffer may have been over 6 months since you added RNase A powder.** If it has been over 6 months since adding the RNase A powder to the PA1 Buffer, the RNase A may not work properly. Add more RNase A powder up to 100 ng/μl.
Appearance of unexpected bands following gel electrophoresis	• **There may have been contamination of chromosomal DNA.** Avoid vortexing the samples vigorously during neutralization step. Lysis time should not exceed 5 minutes. Both will result in shearing of genomic DNA. So, handle the lysate gently.
Sample floating upon loading in an agarose gel	• **Sample may contain ethanol.** Floating is caused by remaining ethanol. Dry the column completely by centrifugation and make sure that no droplet is

	hanging from tip of the column.
Appearance of too many background bands in sequencing analysis	• Check the endonuclease activity of your host *E. coli* strain. HB101, JM series, and normal wild-type hosts that have high endonuclease activity interrupt the sequencing reaction by degrading the plasmid. We recommend using the *end*A- strain instead of *end*A+ strain.

Ordering Information

Description		Cat. No
AccuPrep® Plasmid Mini Extraction Kit	50 reactions	K-3030-1
	200 reactions	K-3030

Related Products

Description	Cat. No
RNase A Powder	KB-0101

Explanation of Symbols

LOT Batch Code	Consult Instructions For Use	RUO Research Use Only	Caution
Biological Risks	Contains Sufficient for <n> tests	Temperature Limitation	Manufacturer
REF Catalog Number	Do not Re-use	Use-by Date	

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