USER GUIDE

AccuPrep® Nano-Plus Plasmid Mini/Midi/Maxi Extraction Kit

Cat. No. K-3111
K-3112
K-3122
K-3131
K-3132

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AccuPrep® Nano-Plus Plasmid Mini/Midi/Maxi Extraction Kit

Kit for the extraction of plasmid from bacterial culture

User Guide

K-3112	K-3111	K-3122	K-3132	K-3131
Σ 50	Σ 200	Σ 25	Σ 10	Σ 25

Version No.: 7 (2022-09-01)

Please read all the information in booklet before using the unit

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Intended Use

AccuPrep® Nano-Plus Plasmid Mini/Midi/Maxi 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

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.

Contents

Product Information

Components

Components	Mini	
	K-3111 (200 reactions)	**K-3112** (50 reactions)
PNA1 Buffer (Resuspension)	60 ml	15 ml
P2 Buffer (Lysis)	60 ml	15 ml
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 x 2 ea
EA Buffer (Elution)	25 ml	15 ml
BST Solution	40 ml	10 ml
RNase A powder, lyophilized	6 mg	1.5 mg
AccuPrep® Binding Column-II plus	200 ea	50 ea
Collection Tube (Filtration)	200 ea	50 ea
One Page Protocol	1 ea	1 ea

Components	Midi	Maxi	
	K-3122 (25 reactions)	**K-3131** (25 reactions)	**K-3132** (10 reactions)
PNA1 Buffer (Resuspension)	100 ml	240 ml	100 ml
P2 Buffer (Lysis)	110 ml	240 ml	110 ml
PA3 Buffer (Neutralization)	100 ml	240 ml	100 ml
W2 Buffer (Washing)	200 ml x 3 ea	400 ml x 3 ea	150 ml x 3 ea
EA Buffer (Elution)	60 ml	60 ml	25 ml
BST Solution	40 ml	40 ml	40 ml
RNase A powder, lyophilized	12 mg	24 mg	12 mg
Clearing Syringe Filter (10 ml)	25 ea	-	-
Clearing Syringe Filter (30 ml)	-	25 ea	10 ea
50 ml Test Tube	25 ea	25 ea	10 ea
DNA Binding Filter Tube (Midi)	25 ea	-	-
DNA Binding Filter Tube (Maxi)	-	25 ea	10 ea
One Page Protocol	1 ea	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). PNA1 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® Nano-Plus Plasmid Extraction Kit	K-3111, K-3112	K-3122	K-3131, K-3132
Starting Culture Volume	1-10 ml	25-75 ml	100-150 ml
Turnaround Time	< 10 min	< 40 min	< 60 min
Column Binding Capacity	up to 30 µg	up to 100 µg	up to 500 µg
Elution Volume	50-100 µl	1 ml	1-2 ml
Typical DNA Yield	up to 20 µg	up to 100 µg	up to 500 µg
DNA Purity	A_{260}/A_{280} > 1.8	A_{260}/A_{280} > 1.8	A_{260}/A_{280} > 1.8
Scale	Mini	Midi	Maxi
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.

Introduction

Product Description

AccuPrep® Nano-Plus Plasmid Extraction Kit is designed for extraction of highly purified plasmid DNA from cultured bacterial cells within 10 minutes (Mini), 40 minutes (Midi), or 60 minutes (Maxi). The kit is based on alkaline lysis (Birnboim *et al*., 1979) of bacterial cells and BIONEER's patented Nano-Technology to effectively remove cell debris particles and proteins. The kit employs *AccuPrep*® Binding Column-II plus (Mini) or DNA Binding Filter Tube (Midi/Maxi) 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 (Mini), 100 μg (Midi), or 500 μg (Maxi) 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 containing nano-particles 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 plus (Mini) or DNA Binding Filter Tube (Midi/Maxi). The high salt condition causes denatured proteins, genomic DNA, and cell debris to form insoluble aggregates, while the plasmid DNA renatures in solution. BIONEER's nano-particles effectively bind to the insoluble aggregates and increases total weight of complexes. 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 elution buffer or nuclease-free water.

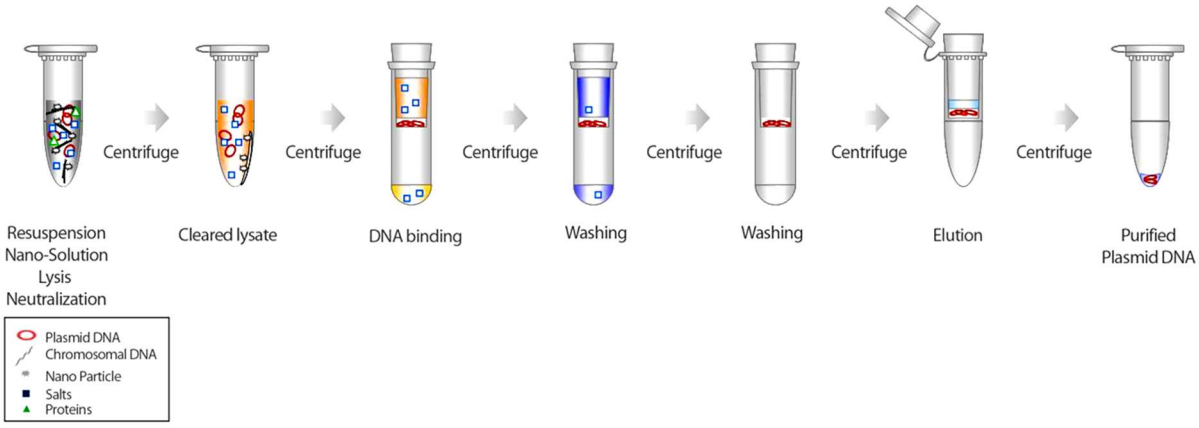
Features & Benefits

- Novel: A new concept of plasmid DNA extraction kit applied with BIONEER's patented Nano-Technology to effectively remove cell debris particles and proteins.
- 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 plasmid DNA is ready-to-use for various application.
- Minimized DNA damage: DNA damage is minimized by avoiding precipitation and use of organic solvents.

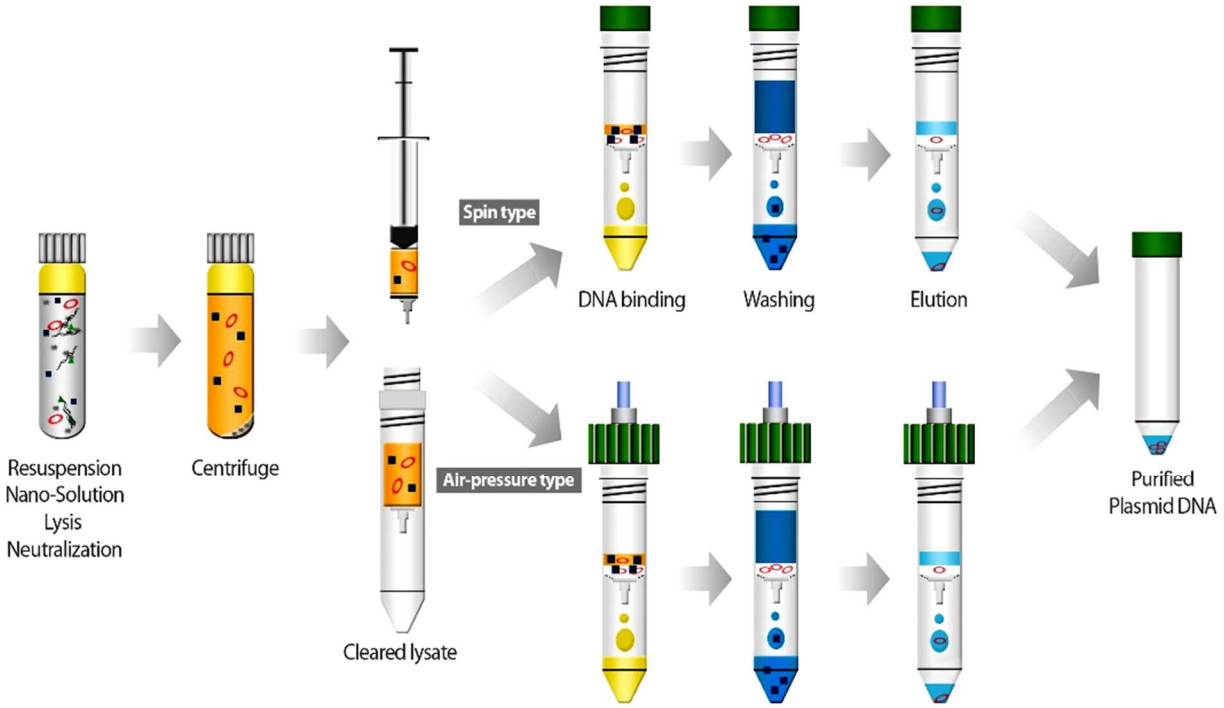
Experimental Procedures

Procedure Overview

- ***AccuPrep*® Nano-Plus Plasmid Mini Extraction Kit**



- ***AccuPrep*® Nano-Plus Plasmid Midi/Maxi Extraction Kit**



Before You Begin

Before proceeding, please check the following:

1. Add RNase A powder to PNA1 Buffer and completely dissolve it. After adding RNase A powder, PNA1 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. Where applicable, add volume of absolute ethanol (not provided) indicated below to PB Buffer.

Cat. No.	K-3111	K-3112
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: $rcf = 1.12 \times r \times (rpm/1,000)^2$

   *** 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 or your established media. 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: 1-5 ml of *E. coli* cells
   - For low-copy number plasmid DNA: 1-10 ml 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 PNA1 Buffer† by vortexing or pipetting.
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.
   † PNA1 Buffer contains nano-particles, please shake well before use.

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 1 minute at >13,000 rpm (16,000 x *g*) at 4°C† in a microcentrifuge.
   * **Note:** After centrifugation, white protein aggregates and nano-particle complexes 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. Apply 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, Y10 series, BMH71-18, and ES1301, which have high endonuclease activity. Denaturation step is not required for the DH5α, XL1-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 8).

Plasmid Midi Extraction

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

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

2. Re-inoculate with overnight cultures diluted 1:1,000 in the 25-50 ml of same media as step 1. Incubate at 37°C for 12-16 hours with shaking.
   * **Note:** Generally, 50 ml of cultures are adequate for purification of low-copy plasmid DNA while 25 ml of cultures are adequate for purification of high-copy plasmid DNA.

3. Harvest cultured cells by centrifugation at 6,000 rpm for 10 minutes at 4°C or 3,500 rpm for 15 minutes at 4°C to pellet cells. Discard the supernatant completely.

- **Cleared lysate preparation**

4. Resuspend the cell pellet from step 3 in 3 ml of PNA1 Buffer† by vortexing or pipetting.
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.
   † PNA1 Buffer contains nano-particles, please shake well before use.

5. Add 3 ml of P2 Buffer to the sample and mix gently by inverting the tube 5-7 times. Incubate for 5 minutes at room temperature.
   * **Note:** Vortexing should be avoided because this will cause shearing of genomic DNA and contamination of plasmid DNA with genomic DNA.

6. Add 3 ml of PA3 Buffer and mix immediately and thoroughly by inverting the tube 5-7 times. Incubate on ice for 5 minutes.
   * **Note:** Be cautious not to shear genomic DNA.

7. Centrifuge the tube at 13,000 rpm for 5 minutes at 4°C†.
   * **Note:** After centrifugation, white protein aggregates and nano-particle complexes 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.

8. Apply 1 ml of BST Solution to the DNA Binding Filter Tube.

9. Centrifuge at 4,500 rpm for 5 minutes at room temperature and discard the flow through.

10. Load the cleared lysate from step 7 into the Clearing Syringe Filter and place the nozzle of Clearing Syringe Filter over the mouth of the DNA Binding Filter Tube.

11. Place the plunger in the Clearing Syringe Filter and push the solution carefully. Collect the filtrate in the DNA Binding Filter Tube.

- **Plasmid DNA Purification (Spin Protocol)**

12. Centrifuge at 3,500 rpm for 3 minutes at room temperature and discard the flow through.

13. Wash the DNA Binding Filter Tube by adding 10 ml of W2 Buffer.

14. Centrifuge at 3,500 rpm for 3 minutes at room temperature and discard the flow through.

15. Repeat steps 13-14.

16. Centrifuge once more at 3,500 rpm for 5 minutes at room temperature to remove residual ethanol completely.

17. Place the DNA Binding Filter only to a new 50 ml Test Tube (supplied with a kit). Add 1 ml of EA Buffer or nuclease-free water to elute DNA and let stand for at least 5 minutes. Centrifuge at 3,500 rpm for 5 minutes at room temperature.

*** 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 8).

- **Plasmid DNA Purification (Air-Pressure Protocol)**

12. Assemble the AccuCap (not provided, Cat. No. KC-1000) to the DNA Binding Filter. Place it on the waste bottle (not provided).

13. Turn on the air pressure system until the filtrate completely passes through.

14. Open the AccuCap and wash the DNA Binding Filter by adding 10 ml of W2 Buffer. Re-assemble the AccuCap with DNA Binding Filter.

15. Turn on the air pressure system until the W2 Buffer completely passes through.

16. Repeat steps 14-15.

17. Turn on the air pressure system once more for 5 minutes to remove residual ethanol completely.

18. Open the AccuCap and add 1 ml of EA Buffer or nuclease-free water to elute DNA. Let stand for at least 5 minutes. Re-assemble the AccuCap with DNA Binding Filter and place the nozzle of DNA Binding Filter at inner of a new 50 ml Test Tube (supplied with a kit).
    * **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 8).

19. Turn on the air pressure system until the plasmid DNA completely passes through.
    * **Note:** Warning! Sometimes plasmid DNA is spattered outside of a 50 ml conical tube. Carefully collect the purified plasmid DNA into the 50 ml conical tube.

Plasmid Maxi Extraction

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

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

2. Re-inoculate with overnight cultures diluted 1:1,000 in the 100-150 ml of same media as step 1. Incubate at 37°C for 12-16 hours with shaking.
   * **Note:** Generally, 150 ml of cultures are adequate for purification of low-copy plasmid DNA while 100 ml of cultures are adequate for purification of high-copy plasmid DNA.

3. Harvest cultured cells by centrifugation at 6,000 rpm for 15 minutes at 4°C or 3,500 rpm for 20 minutes at 4°C to pellet cells. Discard the supernatant completely.

- **Cleared lysate preparation**

4. Resuspend the cell pellet from step 3 in 6 ml of PNA1 Buffer† by vortexing or pipetting.
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.
   † PNA1 Buffer contains nano-particles, please shake well before use.

5. Add 6 ml of P2 Buffer to the sample and mix gently by inverting the tube 5-7 times. Incubate for 5 minutes at room temperature.
   * **Note:** Vortexing should be avoided because this will cause shearing of genomic DNA and contamination of plasmid DNA with genomic DNA.

6. Add 6 ml of PA3 Buffer and mix immediately and thoroughly by inverting the tube 5-7 times. Incubate on ice for 10 minutes.
   * **Note:** Be cautious not to shear genomic DNA.

7. Centrifuge the tube at 13,000 rpm for 10 minutes at 4°C†.
   * **Note:** After centrifugation, white protein aggregates and nano-particle complexes 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.

8. Apply 1 ml of BST Solution to the DNA Binding Filter Tube.

9. Centrifuge at 4,500 rpm for 5 minutes at room temperature and discard the flow through.

10. Load the cleared lysate from step 7 into the Clearing Syringe Filter and place the nozzle of Clearing Syringe Filter over the mouth of the DNA Binding Filter Tube.

11. Place the plunger in the Clearing Syringe Filter and push the solution carefully. Collect the filtrate in the DNA Binding Filter Tube.

- **Plasmid DNA Purification (Spin Protocol)**

12. Centrifuge at 3,500 rpm for 5 minutes at room temperature and discard the flow through.

13. Wash the DNA Binding Filter Tube by adding 20 ml of W2 Buffer.

14. Centrifuge at 3,500 rpm for 5 minutes at room temperature and discard the flow through.

15. Repeat steps 13-14.

16. Centrifuge once more at 3,500 rpm for 5 minutes to remove residual ethanol completely.

17. Place the DNA Binding Filter only to a new 50 ml Test Tube (supplied with a kit). Add 1 ml of EA Buffer or nuclease-free water to elute DNA and let stand for at least 5 minutes. Centrifuge at 3,500 rpm for 5 minutes at room temperature.

*** 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 8).

- **Plasmid DNA Purification (Air-Pressure Protocol)**

12. Assemble the AccuCap (not provided, Cat. No. KC-1000) to the DNA Binding Filter. Place it on the waste bottle (not provided).

13. Turn on the air pressure system until the filtrate completely passes through.
14. Open the AccuCap and wash the DNA Binding Filter by adding 20 ml of W2 Buffer. Re-assemble the AccuCap with DNA Binding Filter.

15. Turn on the air pressure system until the W2 Buffer completely passes through.

16. Repeat steps 14-15.

17. Turn on the air pressure system once more for 5 minutes to remove residual ethanol completely.

18. Open the AccuCap and add 1 ml of EA Buffer or nuclease-free water to elute DNA. Let stand for at least 5 minutes. Re-assemble the AccuCap with DNA Binding Filter and place the nozzle of DNA Binding Filter at inner of a new 50 ml Test Tube (supplied with a kit).
    * **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 8).

19. Turn on the air pressure system until the plasmid DNA completely passes through.
    * **Note:** Warning! Sometimes plasmid DNA is spattered outside of a 50 ml conical tube. Carefully collect the purified plasmid DNA into the 50 ml conical tube.

Troubleshooting

Problem	Comments
Low plasmid DNA yield	• **You may have used too much culture.** Too much culture causes incomplete lysis and neutralization and Clearing Syringe Filter may clog during filtration.
	• **The cells may not have been completely resuspended with PNA1 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.
	• **PNA1 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 PNA1 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® Nano-Plus Plasmid Mini Extraction Kit	50 reactions	K-3112
	200 reactions	K-3111
AccuPrep® Nano-Plus Plasmid Midi Extraction Kit	25 reactions	K-3122
AccuPrep® Nano-Plus Plasmid Maxi Extraction Kit	10 reactions	K-3132
	25 reactions	K-3131

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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