ISO 9001
Certified

Ver. 2.0

User's Guide

Global Genomics Partner

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

Cat. No. K-3111

Cat. No. K-3112

Cat. No. K-3122

Cat. No. K-3131

Cat. No. K-3132

BIONEER
bioneer corporation

Safety Warnings and Precautions.

AccuPrep® Nano-Plus Plasmid Extraction Kit is developed and sold for research purposes only. It is not recommended for human or animal diagnostic use, unless cleared for such purposes by the appropriate regulatory authorities in the country of use.

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 Sheets (MSDS).

The Neutralization Buffer contains a chaotrophic salt and should be handled with care. Chaotrophic salts form highly reactive compounds when combined with bleach. Thus great care must be taken to properly dispose of this solution.

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 Bioneer's quality management system from product development to production to quality assurance and supplier qualification meets or exceeds the world-class quality standards.

Trademarks

AccuPrep® is trademark of Bioneer Corporation in Korea.

***AccuPrep*® Nano-Plus Plasmid Mini Extraction Kit (K-3111, K-3112)**

***AccuPrep*® Nano-Plus Plasmid Midi Extraction Kit (K-3122)**

***AccuPrep*® Nano-Plus Plasmid Maxi Extraction Kit (K-3131, K-3132)**

AccuCap (KC-1000)

CONTENTS

AccuPrep® Nano-Plus Plasmid Extraction Kit *Technical Manual*

I. Description

The *AccuPrep*® Nano-Plus Plasmid Mini, Midi and Maxi Extraction Kit was developed for the extraction of highly purified plasmid DNA from cultured bacterial cells within 10 min.(mini), 40 min.(Midi) or 60 min.(Maxi) The overall principle combines modified alkaline lysis method(Birnboim *et al*, 1979) and Bioneer's novel Nano-Technology(Patent pending). Collected cells are re-suspended in Resuspension Buffer contains Nano-solution. Following the addition of Lysis Buffer and Neutralization Buffer to the lysate, the chromosomal DNA and cell debris will be forms an insoluble aggregate. Bioneer's Nano-solution effectively bound to the protein aggregate and increase the total weight of the complex. The insoluble protein aggregate is removed following centrifugation and transfer the clear lysate to the DNA binding filter tube. The cleared lysate contains a chaotropic salt originating from Neutralization Buffer which helps the binding of the plasmid DNA on the membrane surface. The DNA binding filter which is packed with silica based membrane has enough surface area to bind up to 20 μg (mini), 100 μg (Midi) or 500 μg (Maxi) of plasmid DNA. Any salts and precipitates are eliminated by addition of the Washing Buffer. Finally, highly purified plasmid DNA can be eluted with Elution Buffer or Nuclease free autoclaved distilled water(not provided).

II. Kit Components

	Mini	
Cat. No	**K-3111**	**K-3112**
Buffer ①	60 ml	15 ml
Buffer ②	60 ml	15 ml
Buffer ③	80 ml	20 ml
Buffer D	75 ml	18 ml
Buffer ④	2×16 ml	2×4 ml
Buffer ⑤	24 ml	6 ml
RNase A powder	6 mg	1.5 mg
DNA binding column tube	200 ea	50 ea
User's Guide	1 ea	1 ea
One Page Protocol	1 ea	1 ea

	Midi	Maxi	
Cat. No	**K-3122**	**K-3131**	**K-3132**
Buffer ①	90 ml	240 ml	96 ml
Buffer ②	90 ml	240 ml	96 ml
Buffer ③	90 ml	240 ml	96 ml
Buffer ④	3×40 ml	3×80 ml	3×30 ml
Buffer ⑤	50 ml	50 ml	20 ml
RNase A powder	9 mg	24 mg	9.6 mg
Clearing Syringe Filter (10 ml)	25 ea	-	-
Clearing Syringe Filter (30 ml)	-	25 ea	10 ea
DNA Binding Filter Tubes (Midi)	25 ea	-	-
DNA Binding Filter Tubes (Maxi)	-	25 ea	10 ea
User's Guide	1 ea	1 ea	1 ea
One Page Protocol	1 ea	1 ea	1 ea

※ Buffer ③ and ④ contains chaotropic salt and should be handled with care. Chaotrophic salts can makes highly reactive compounds when mixed with disinfecting agent such as bleach.

※ All buffers and DNA binding columns can be stored under room temp. But, Buffer ① must stored at 4℃ after addition of RNase A powder.

III. Before You Begin

Before you start your prep, please check the followings.

1. Chemicals

- Did you add RNase A powder to Buffer ① and completely dissolve it ?
- Did you add absolute EtOH to Buffer D as described below ?
- Did you add absolute EtOH to Buffer ④ as described below ?

Cat. No.	K-3111	K-3112
Buffer D	75 ml	18 ml
Absolute EtOH	45 ml	10.8 ml
Total	120 ml	28.8 ml

Cat. No.	K-3111	K-3112	K-3122	K-3131	K-3132
Buffer ④	16 ml	4 ml	40 ml	80 ml	30 ml
Absolute EtOH	64 ml	16 ml	160 ml	320 ml	120 ml
Total	80 ml	20 ml	200 ml	400 ml	150 ml

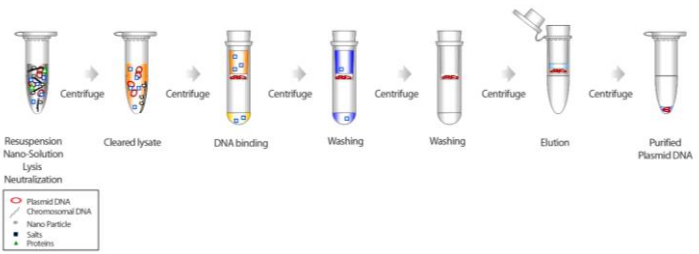
2. Equipments

- High speed refrigerated centrifuge (capable of ≥12,000 rpm, 4℃, e.g., Beckman Avanti® J Series, Sorvall® RC-5B Plus, Hanil Supra series)
- Swing Bucket rotor type Table-top centrifuge (capable of ≥3,000 rpm, e.g., Beckman Allegra® X-12 Series, Sorvall Legend Mach 1.6/R Tabletop, Hanil Union 32 R Plus)
- Air-pressure system (capable of at least 40 psi, 2.81kg/cm^2, 2.76 bar) or Oil-less(or Oil free) Air pump(or compressor)

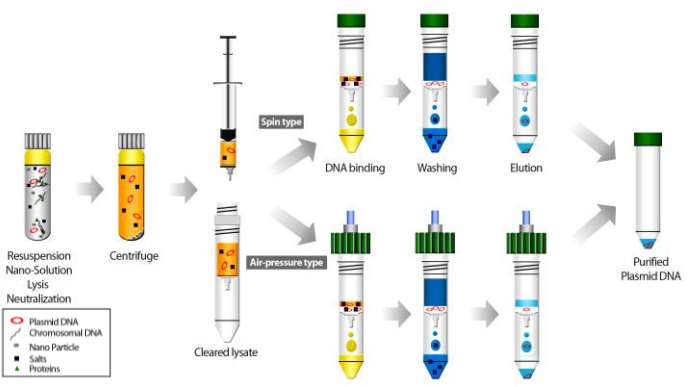
Are you ready to prep your Plasmid DNA?

IV. Procedure

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



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



V. Experimental Protocol for Mini Prep.

■ ***E.coli*** **cell preparation**

1. **Pick up a single colony from fresh cultured LB(Luria-Bertani) agar plate(contains antibiotics) or your established media and inoculate the cell into the 1-5 ml of fresh LB liquid media (contains antibiotics) or your established media at 37℃ with shaking for 12-16 hr.**
   Do not over-growth your *E.coli* cell. It will decrease the productivity because of the 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. **Collect the *E.coli* cells by centrifugation at >8,000 rpm for 2 min. or >3,000 rpm for 5 min. And completely remove of the media by pipetting.**

■ **Cleared lysate preparation**

1. **Add 250 µl of Buffer ① to the collected cells and completely resuspend by vortexing or pipetting.**
   Complete resuspension will make high lysis efficiency. Buffer ① contains Nano-particle, please shake well before use.

2. **Add 250 µl of Buffer ② and mix by inverting the tube 3-4 times gently.**
   Avoid vortex! Vortexing may cause shearing of genomic DNA. It is important to invert gently.

3. **Add 350 µl of Buffer ③ and immediately mix by inverting the tube 3-4 times, gently.**
   Genomic DNA and cell debris will form an insoluble complex. Again, be cautions not to shear genomic DNA.

4. **Centrifugation the tube at 13,000 rpm, 4℃ for 1 min. (>16,600 ×g) in a micro-centrifuge.**
   After centrifugation, white protein aggregate and Nano-particle complex will appear at the bottom of the tube. If your centrifuge is not enough to get a cleared lysate, please centrifuge again.

■ **Plasmid DNA Purification**

5. **Transfer the cleared lysate to the DNA binding column tube and centrifuge at 13,000 rpm for 1 min. Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0 ml collection tube.**

6. **(Optional) Add 500 µl of Buffer D and wait for 5 min. and centrifuge at 13,000 rpm for 1 min. Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0 ml collection tube.**
   This step is required if you are using an *endA*$^+$ strains which has a high endonuclease activity. BL21, CJ236, HB101, JM83, JM 101, JM110, LE392, NM series strains, PR series strains, Q358, PR1, TB1, TG1, Y10 series strains, BMH71-18 and ES1301 are *endA*$^+$ strains, thus they produce highly active endonucleases that can degrade plasmids. 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.

7. **Add 700 µl of Buffer ④ to the DNA binding column tube and centrifuge at 13,000 rpm for 1 min. Pour off the flow-through and re-assemble the DNA binding filter column with the 2.0 ml collection tube.**
   This removes salts and soluble debris. The amount of plasmid washed away in 80 % ethanol is negligible.

8. **Dry by additional centrifugation at 13,000 rpm for 1 min. to remove the residual ethanol.**

9. **Transfer the DNA binding filter column to the new 1.5 ml microcentrifuge tube (not provided).**

10. **Add 50-100 µl of Buffer ⑤ to the DNA binding filter column, and wait for at least 1 min. for elution.**
    If you want to get a more concentrated solution of plasmid, a smaller volume is appropriate, but total yield may be reduced. If the plasmid is low copy or larger than 10 kb, the yield of plasmid may not be sufficient. Pre-warmed (about 60 ℃) elution buffer will improve efficiency of elution.

11. **Elute the plasmid DNA by centrifugation at 13,000 rpm for 1 min.**
    If you want more quantity, elute the sample twice and use after concentrating process.

VI. Experimental Protocol for Midi prep.

■ *E.coli* cell preparation

1. **Pick up a single colony from fresh cultured LB(Luria-Bertani) agar plate(contains antibiotics) or your established media and inoculate the cell into the 5 ml of fresh LB liquid media (contains antibiotics) or your established media at 37℃ with shaking.**

2. **After 12-16 hr, take 50 ul of cultured cell and re-inoculate the cells into the 25 ~ 50 ml of fresh LB liquid media (contains antibiotics) or your established media at 37℃ with shaking for 12 - 16 hr.**

■ Cleared Lysate Preparation

(This step needs high speed refrigerated centrifuge, e. g., Beckman Avanti® J series, Sorvall® RC-5B Plus, Hanil Supra series)

1. **Harvest 25 ml (high copy plasmid) or 50 ml (low copy plasmid) of cultured *E. coli* cells by centrifugation at 6,000 rpm, 4℃ for 10 min. or 3,500 rpm, 4℃ for 15 min. and completely remove the media.**

2. **Add 3 ml of Buffer ① to the collected cells and completely resuspend by vortexing or pipetting.**
   Complete resuspension will make high lysis efficiency. Buffer ① contains Nano-particle, please shake well before use.

3. **Add 3 ml of Buffer ② and mix by inverting the tube 5-7 times gently, and incubate the centrifuge tube at room temp. for 5 min.**
   Avoid vortexing! Vortexing may cause shearing of genomic DNA. It is important to invert gently.

4. **Add 3 ml of Buffer ③ and immediately mix by inverting the tube 5-7 times gently, and incubate the centrifuge tube on ice for 5 min.**
   Genomic DNA and cell debris will form a white mass. Again, be cautious not to shear genomic DNA.

5. **Centrifuge the tube at 13,000 rpm, 4℃ for 5 min.**
   After centrifugation, white protein aggregate will appear.

6. **Transfer the lysate to the Clearing Syringe Filter.**
   Do not insert the plunger into Clearing Syringe Filter before transferring the cleared lysate. And place the nozzle of the Clearing Syringe Filter over the mouth of the DNA binding filter tube.

7. **Insert the plunger into the Clearing Syringe Filter carefully, and collect the filtrate in the DNA binding filter tube.**

■ Plasmid DNA Purification (Spin Method)

(This method needs a swing bucket rotor type centrifuge, e.g., Allegra® X-12 Series Sorvall Mach 1.6/R Tabletop, Hanil 32R series)

8. **Centrifuge the DNA binding filter tube at 3,500 rpm, room temp. for 3 min.**

9. **Pour off the flow-through and re-assemble the DNA binding filter with the 50 ml test tube.**

10. **Add 10 ml of Buffer ④ to the DNA binding filter tube and centrifuge at 3,500 rpm, room temp. for 3 min.**

11. **Pour off the flow-through and re-assemble the DNA binding filter with the 50 ml test tube.**
    This step removes salts and soluble debris. The amount of plasmid washed away in 80 % ethanol is negligible.

12. **Repeat Step 10. and 11.**

13. **Dry the DNA binding filter by additional centrifuge at 3,500 rpm, room temp. for 5 min. to remove the residual ethanol.**

14. **Transfer the DNA binding filter to the new 50 ml test tube (not provided).**

15. **Add 1 ml of Buffer ⑤ to center of the DNA binding filter, and wait for at least 5 min. for elution.**
    If, more concentrated solution of plasmid is required, a smaller volume can be used, but total yield may be reduced. Also if the plasmid is low copy or larger than 10 kb, the yield of plasmid may not be sufficient. Pre-warmed (about 60℃) elution buffer will also improve efficiency of elution.

16. **Elute the plasmid DNA by centrifuge at 3,500 rpm, room temp. for 5 min.**
    If you want more quantity, elute the sample twice and use after concentrating process.

■ Plasmid DNA Purification (Air-Pressure Method)

(This method needs at least 40 psi, 2.81kg/cm^2 or 2.76 bar of air-pressure system or air pump, air compressor)

8. **Assemble the AccuCap(not provided, Cat. No. KC-1000) to the DNA binding filter and locate above on the waste bottle (not provided).**

9. **Turn on the air pressure system until completely pass the filtrate, carefully.**

10. **Open the AccuCap and add 10 ml of Buffer ④ to the DNA binding filter and re-assemble the AccuCap with DNA binding filter.**

11. **Turn on the air pressure system until completely pass the washing buffer, carefully.**

12. **Repeat Step 10. and 11.**
    This step removes of the salts and soluble debris. The amount of plasmid washed away in 80 % ethanol is negligible.

13. **Dry the DNA binding filter by additional air injection for 5 min. to remove the residual ethanol.**

14. **Open the AccuCap and add 1 ml of Buffer ⑤ to center of the DNA binding filter, and wait for 5 min. for elution.**
    If you want to get a more concentrated solution of plasmid, a smaller volume is appropriate, but total yield may be reduced. If the plasmid is low copy or larger than 10 kb, the yield of plasmid may not be sufficient. Pre-warmed (about 60℃) elution buffer will improve efficiency of elution.

15. **Re-assemble the AccuCap with DNA binding filter and locate the nozzle of the DNA binding filter at inner side of the new 50 ml test tube (not provided).**

16. **Elute the plasmid DNA by air injection to the DNA binding filter until completely pass the buffer.**
    Warning! Sometimes Plasmid DNA solution spattered out side of the 50 ml test tube. Carefully collect the purified Plasmid DNA into the 50 ml test tube.

VII. Experimental Protocol for Maxi prep.

■ *E.coli* cell preparation

1. **Pick up a single colony from fresh cultured LB(Luria-Bertani) agar plate(contains antibiotics) or your established media and inoculate the cell into the 5 ml of fresh LB liquid media (contains antibiotics) or your established media at 37℃ with shaking.**

2. **After 12-16 hr, take 100 ul of cultured cell and re-inoculate the cells into the 100 ~ 150 ml of fresh LB liquid media (contains antibiotics) or your established media at 37℃ with shaking for 12 - 16 hr.**

■ Cleared Lysate Preparation

(This step needs high speed refrigerated centrifuge, e. g., Beckman Avanti® J series, Sorvall® RC-5B Plus, Hanil Supra series)

1. **Harvest 100 ml (high copy plasmid) or 150 ml (low copy plasmid) of cultured *E. coli* cells by centrifugation at 6,000 rpm, 4℃ for 15 min. or 3,500 rpm, 4℃ for 20 min. and completely remove the media.**

2. **Add 9 ml of Buffer ① to the collected cells and completely resuspend by vortexing or pipetting.**
   Complete resuspension will make high lysis efficiency. Buffer ① contains Nano-particle, please shake well before use.

3. **Add 9 ml of Buffer ② and mix by inverting the tube 5-7 times gently, and incubate the centrifuge tube at room temp. for 5 min.**
   Avoid vortexing! Vortexing may cause shearing of genomic DNA. It is important to invert gently.

4. **Add 9 ml of Buffer ③ and immediately mix by inverting the tube 5-7 times gently, and incubate the centrifuge tube on ice for 10 min.**
   Genomic DNA and cell debris will form a white mass. Again, be cautious not to shear genomic DNA.

5. **Centrifuge the tube at 13,000 rpm, 4℃ for 10 min.**
   After centrifugation, white protein aggregate will appear.

6. **Transfer the lysate to the Clearing Syringe Filter.**
   Do not insert the plunger into Clearing Syringe Filter before transferring the cleared lysate. And place the nozzle of the Clearing Syringe Filter over the mouth of the DNA binding filter tube.

7. **Insert the plunger into the Clearing Syringe Filter carefully, and collect the filtrate in the DNA binding filter tube.**

■ Plasmid DNA Purification (Spin Method)

(This method needs a swing bucket rotor type centrifuge, e.g., Allegra® X-12 Series Sorvall Mach 1.6/R Tabletop, Hanil 32R series)

8. **Centrifuge the DNA binding filter tube at 3,500 rpm, room temp. for 5 min.**

9. **Pour off the flow-through and re-assemble the DNA binding filter with the 50 ml test tube.**

10. **Add 20 ml of Buffer ④ to the DNA binding filter tube and centrifuge at 3,500 rpm, room temp. for 5 min.**

11. **Pour off the flow-through and re-assemble the DNA binding filter with the 50 ml test tube.**
    This step removes salts and soluble debris. The amount of plasmid washed away in 80 % ethanol is negligible.

12. **Repeat Step 10. and 11.**

13. **Dry the DNA binding filter by additional centrifuge at 3,500 rpm, room temp. for 5 min. to remove the residual ethanol.**

14. **Transfer the DNA binding filter to the new 50 ml test tube (not provided).**

15. **Add 1 ml of Buffer ⑤ to center of the DNA binding filter, and wait for at least 5 min. for elution.**
    If, more concentrated solution of plasmid is required, a smaller volume can be used, but total yield may be reduced. Also if the plasmid is low copy or larger than 10 kb, the yield of plasmid may not be sufficient. Pre-warmed (about 60℃) elution buffer will also improve efficiency of elution.

16. **Elute the plasmid DNA by centrifuge at 3,500 rpm, room temp. for 5 min.**
    If you want more quantity, elute the sample twice and use after concentrating process.

■ Plasmid DNA Purification (Air-Pressure Method)

(This method needs at least 40 psi, 2.81kg/cm^2 or 2.76 bar of air-pressure system or air pump, air compressor)

8. **Assemble the AccuCap(not provided, Cat. No. KC-1000) to the DNA binding filter and locate above on the waste bottle (not provided).**

9. **Turn on the air pressure system until completely pass the filtrate, carefully.**

10. **Open the AccuCap and add 20 ml of Buffer ④ to the DNA binding filter and re-assemble the AccuCap with DNA binding filter.**

11. **Turn on the air pressure system until completely pass the washing buffer, carefully.**

12. **Repeat Step 10. and 11.**
    This step removes of the salts and soluble debris. The amount of plasmid washed away in 80 % ethanol is negligible.

13. **Dry the DNA binding filter by additional air injection for 5 min. to remove the residual ethanol.**

14. **Open the AccuCap and add 1 ml of Buffer ⑤ to center of the DNA binding filter, and wait for 5 min. for elution.**
    If you want to get a more concentrated solution of plasmid, a smaller volume is appropriate, but total yield may be reduced. If the plasmid is low copy or larger than 10 kb, the yield of plasmid may not be sufficient. Pre-warmed (about 60℃) elution buffer will improve efficiency of elution.

15. **Re-assemble the AccuCap with DNA binding filter and locate the nozzle of the DNA binding filter at inner side of the new 50 ml test tube (not provided).**

16. **Elute the plasmid DNA by air injection to the DNA binding filter until completely pass the buffer.**
    Warning! Sometimes Plasmid DNA solution spattered out side of the 50 ml test tube. Carefully collect the purified Plasmid DNA into the 50 ml test tube.

VIII. Troubleshooting

1. Low yield of plasmid

1) Did you harvest a sufficient amount of cells ? The yield is dependent on the host strain type and an overload of cells may decrease the yield.

2) Did you completely resuspend the cells with Buffer ① ? Incomplete resuspension decreases the efficiency of lysis.

3) Is there precipitated salt in the Buffer ③ ? Vortex or shake well to re-dissolve the precipitant. An improper concentration of the chaotrophic agent will decrease the yield. If it does not re-dissolve easily, warm it to 60 ℃.

4) Has it been over 6 months since you added RNase A powder? Low concentration of RNase A can result in a low yield of plasmid. After about 6 months, add more RNase A, up to 100 μg/μl.

2. Contamination of chromosomal DNA (The appearance of unexpected bands following gel electrophoration).

During neutralization step, samples should not be vortex or shaken vigorously. Also, the period of lysis should not be longer than 5 min. Both can shear the chromosomal DNA. Handle the lysate gently!

3. Sample floats upon loading in agarose gel.

Sample contains alcohol. Floating is caused by leftover ethanol. You must always dry the column completely by centrifugation and make sure that no droplet is hanging from the tip of the binding column.

4. Too many background bands appear in sequencing analysis.

Did you check the endonuclease activity of your strain of host *E. coli*? 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 *EndA*$^-$ strain instead of *EndA*$^+$ strain.

5. Sample contains RNA.

1) RNase activity is weakened. If it has been over 6 months since adding the RNase A powder to the Resuspension, the RNase A may not work properly. Add more RNase A powder, up to 100 μg/μl.

IX. 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. Taylor, R. G., Walker, D. C. and Mclnnes, R. R. (1993) *Nucleic Acids Res*., **21**, 1677-1678

4. Melzak, K. A. *et al*. (1996), *J Colloid and Interface Sci*., **181**, 635-644

5. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning* 2nd Ed.