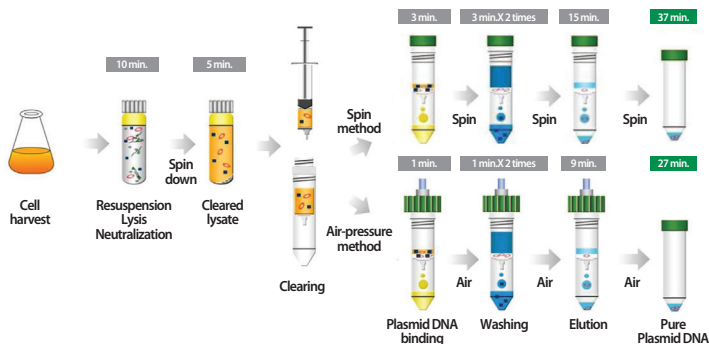


AccuPrep® Nano-Plus Plasmid Midi Extraction Kit (K-3122)

I Before You Begin

1) Did you add RNase A powder to PNA1 Buffer?

After adding RNase A, PNA1 Buffer should be stored at 4°C.



II Cleared lysate Preparation

This step needs a high speed refrigerated centrifuge, e.g., Beckman Avanti® J Series, Sorvall® RC-5B PLUS, Hanil Supra Series.

- Harvest the 25 ml (high copy number plasmid) or 50 ml (low copy number plasmid) of cultured *E. coli* cells by centrifugation at 6,000 rpm, 4°C for 10 min or 3,500 rpm, 4°C for 15 min and completely remove the media and completely remove the media by pipetting.
- Add 3 ml of PNA1 Buffer to the collected cells and completely resuspend by vortexing or pipetting (PNA1 Buffer contains Nano-particle. Please shake well before use.).
- Add 3 ml of P2 Buffer and mix by inverting the tube 5~7 times gently, and incubate the centrifuge tube at RT for 5 min.
(Note) Do not vortex but just invert gently. Vortexing can cause shearing of genomic DNA.
- Add 3 ml of PA3 Buffer and immediately mix by inverting the tube 5~7 times gently, and incubate the centrifuge tube on ice for 5 min.
(Caution) Again, be cautious not to shear genomic DNA. Genomic DNA and cell debris will form an insoluble complex.
- Centrifuge the tube at 13,000 rpm, 4°C for 5 min.
- Add 1 ml of BST Solution to the Binding column tube (fit in a collection tube) and centrifuge for 5 min at 4,500 rpm.
- Discard the solution from the collection tube and reuse the collection tube.
- Transfer the lysate to the Clearing Syringe Filter.
- Insert the plunger into the Clearing Syringe Filter carefully, and collect the filtration in the DNA binding filter tube.

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III Plasmid DNA Purification

Here are Plasmid Purification Methods after obtaining a cleared lysate:

A. Spin Method

B. Air-Pressure Method

A. Spin Method

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

- 1) Centrifuge the DNA binding filter tube at **3,500 rpm, RT for 3 min.**
- 2) Pour off the flow-through and re-assemble the DNA binding filter with the 50 ml test tube.
- 3) Add **10 ml of W2 Buffer** to the DNA binding filter tube and **centrifuge at 3,500 rpm, RT for 3 min.**
- 4) Pour off the flow-through and re-assemble the DNA binding filter with the 50 ml test tube.
- 5) **Repeat Step 3~4.**
- 6) Dry the DNA binding filter by additional centrifuge at **3,500 rpm, RT for 5 min** to remove the residual ethanol.
- 7) Transfer the DNA binding filter to the new 50 ml test tube.
- 8) Add **1 ml of EA Buffer** to center of the DNA binding filter, and wait for 5 min.
- 9) Elute the plasmid DNA by centrifuge at **3,500 rpm, RT for 5 min.**

B. Air-Pressure Method

For this method, air-pressure system, air pump or air compressor is required.
(Recommended specification : at least 40 psi, 2.81 kg/cm² or 2.76 bar)

- 1) Assemble the AccuCap (not provided, Cat. No. KC-1000) to the DNA binding filter and locate above on the waste bottle (not provided).
- 2) Turn on the air-pressure system until the filtrate passes through the filter completely.
- 3) Open the AccuCap and **add 10 ml of W2 Buffer** to the DNA binding filter and re-assemble the AccuCap with DNA binding filter.
- 4) Turn on the air-pressure system until the filtrate passes through the filter completely.
- 5) **Repeat Step 3~4.**
- 6) Dry the DNA binding filter by additional air injection for **5 min** to remove the residual ethanol.
- 7) Open the AccuCap and **add 1 ml of EA Buffer** to center of the DNA binding filter, and wait for 5 min.
- 8) 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.
- 9) Elute the plasmid DNA by air injection to the DNA binding filter until completely the buffer passes through the filter completely.

※ For more information, please visit www.bioneer.com and refer to the User's Guide of this kit.