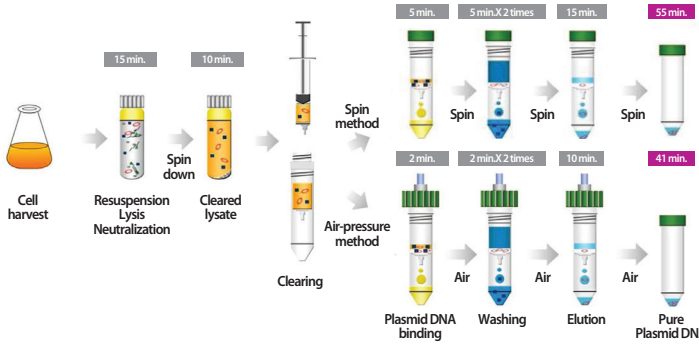
AccuPrep® Nano-Plus Plasmid Maxi Extraction Kit (K-3131, K-3132)

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.

1) Harvest the 100 ml (high copy number plasmid) or 150 ml (low copy number plasmid) of cultured *E. coli* cells by centrifugation at 6,000 rpm, 4˚C for 15 min or 3,500 rpm, 4˚C for 20 min and completely remove the media and completely remove the media by pipetting.
2) Add **6 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.).
3) Add **6 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.
4) Add **6 ml** of **PA3 Buffer** and immediately mix by inverting the tube 5~7 times gently, and incubate the centrifuge tube on ice for **10 min**.
(Caution) Again, be cautions not to shear genomic DNA. Genomic DNA and cell debris will form an insoluble complex.
5) Centrifuge the tube at **13,000 rpm**, **4˚C** for **10 min**.
6) 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.
7) Discard the solution from the collection tube and reuse the collection tube.
8) Transfer the lysate to the Clearing Syringe Filter.
9) Insert the plunger into the Clearing Syringe Filter carefully, and collect the filtration in the DNA binding filter tube.

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

Ⅲ Plasmid DNA Purification

Here are Plasmid Purification Methods after obtaining a cleared lysate:

A. Spin Method

B. Air-Pressure Method

A. Spin Method

1) Centrifuge the DNA binding filter tube at **3,500 rpm**, **RT** for **5 min**.
2) Pour off the flow-through and re-assemble the DNA binding filter with the 50 ml test tube.
3) Add **20 ml** of **W2 Buffer** to the DNA binding filter tube and centrifuge at **3,500 rpm**, **RT** for **5 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/cm^2 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 **20 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.