

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

- 1) Did you add **RNase A** powder to **PNA1 Buffer** and completely dissolve it?
After adding RNase A, PNA1 Buffer should be stored at 4°C.
- 2) Did you add the correct amount of **absolute ethanol** to **PB Buffer**?
- 3) Before starting extraction process, heat the EA Buffer at 56~60°C.
- 4) The protective seal in BST Solution should be completely removed. BST Solution may be discolored, but it does not affect nucleic acid extraction.

II Experimental Protocol

- 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 or your established media at 37°C with shaking for 12~16 hours.
Do not overgrowth *E.coli* cell. It will decrease the productivity because of the 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) 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.
- 3) Add **250 µl** of **PNA1 Buffer** to the collected cells and completely resuspend by vortexing or pipetting. PNA1 Buffer contains Nano-particle. Please shake well before use.
- 4) Add **250 µl** of **P2 Buffer** and mix by inverting the tube 3~4 times gently. **(Caution)** Avoid vortex! Vortexing may cause shearing of genomic DNA. It is important to invert gently.
- 5) Add **350 µl** of **PA3 Buffer** and immediately mix by inverting the tube 3~4 times, gently. **(Caution)** Again, be cautions not to shear genomic DNA. Genomic DNA and cell debris will form an insoluble complex.
- 6) Centrifuge the tube at **13,000 rpm, 4°C for 1 min** in a microcentrifuge. After centrifugation, white protein aggregate and Nano particle complex will appear at the bottom of the tube. **(Option)** If your centrifuge is not enough to get a cleared lysate, please centrifuge again.
- 7) Add **100 µl** of **BST Solution** to the Binding column tube (fit in a collection tube) and centrifuge for **30 sec** at **13,000 rpm**.
- 8) Discard the solution from the collection tube and reuse the collection tube.
- 9) Transfer the cleared lysate to the Binding column (fit in a collection tube) and centrifuge at **13,000 rpm for 1 min**. Pour off the flow-through and re-assemble the Binding column with the collection tube.

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- 10) (Option) Add **500 µl** of **PB Buffer** and wait for 5 min and centrifuge at **13,000 rpm** for **1 min**. Discard the solution from the collection tube and reuse the 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.

- 11) Add **700 µl** of **W2 Buffer** to the Binding column and centrifuge at **13,000 rpm** for **1 min**. Pour off the flow-through and re-assemble the Binding column with the collection tube.

- 12) Centrifuge once more at **13,000 rpm** for **5 min** to remove ethanol completely.

- 13) Transfer the Binding column to a 1.5 ml tube (not provided).

- 14) Add **50~100 µl** of **EA Buffer** to the Binding column, and wait for at least 1 min.

(Option) 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 °C) EA Buffer will improve efficiency of elution.

- 15) Elute the plasmid DNA by centrifugation at **13,000 rpm** for **1 min**.

(Option) If you want more quantity, elute the sample twice.

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