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One page protocol

AccuPrep® Nano-Plus Plasmid Mini Extraction Kit (K-3111G)

Ⅰ Before You Begin

1) Add **RNase A** powder to **PNA1 Buffer** and completely dissolve it. After adding RNase A, PNA1 Buffer should be stored at **4°C.**
2) Add correct amount of **absolute ethanol (not provided)** to **PB Buffer** (see bottle label).
3) Pre-heat EA Buffer at 60°C before use.
4) The protective seal in **BSTB Solution** should be completely removed. **BSTB Solution** may be discolored, but it does not affect nucleic acid extraction.
5) Add correct amount of **absolute ethanol (not provided)** to **WB2 Buffer** and **BSTB Solution**, respectively (see bottle label).

Ⅱ Experimental Protocol

1) Pick up a single colony from either a freshly cultured LB (Luria-Bertani) agar plate (contains antibiotics) or your established media and inoculate the cell into 1-5 ml of fresh LB liquid media or your established media at 37°C with shaking for 12-16 hours.
(Note) Do not overgrow *E. coli* cell. It will decrease the DNA yield due to 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. Discard the supernatant carefully with a pipette.
3) Add **250 µl** of **PNA1 Buffer** to the collected cells and completely resuspend by vortexing or pipetting.
(Note) PNA1 Buffer contains nano-particles, please shake well before use.
4) Add **250 µl** of **P2 Buffer** and mix by gently inverting the tube 3-4 times.
(Note) 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.
(Note) Be cautious 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.
(Note) If your centrifugation is not enough to get a cleared lysate, please centrifuge again.
7) Add **100 µl** of **BSTB Solution** to the Binding column tube (fitted in a collection tube) and centrifuge at **13,000 rpm** for **30 sec.**
8) Discard the solution from the collection tube and reuse the collection tube.
9) Transfer the cleared lysate from step 6) to the Binding column (fitted 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.

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, PR series, Q358, PR1, TB1, TG1, Y10 series, 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 **WB2 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 **1 min** to completely remove residual ethanol.
13) Transfer the Binding column to a clean 1.5 ml tube (not provided).
14) Add **50-100 µl** of **EA Buffer** or nuclease-free water 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 of low copy or larger than 10 kb, total yield of plasmid may not be sufficient. Pre-warmed (about 60 ℃) **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 a higher yield, elute the sample twice.

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