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One page protocol

AccuPrep® Plasmid Mini Extraction Kit (K-3030, K-3030-1)

Ⅰ Before You Begin

1) Did you add **RNase A** powder to **PA1 Buffer** and completely dissolve it? After adding RNase A, PA1 Buffer should be stored at **4℃**.
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℃**.
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℃ with shaking for 12~16 hours.
   Do not over-growth *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 **PA1 Buffer** to the collected cells and completely resuspend by vortexing or pipetting.
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℃** for **10 min** in a microcentrifuge. After centrifugation, white protein aggregate 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 **1 min** to remove ethanol completely.
13) Transfer the Binding column to a1.5 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.