MagListo™ 5M Plasmid Extraction Kit (K-3600, K-3601)

Ⅰ Before You Begin

1) Completely dissolve RNase A powder in PM1 Buffer.
   After adding RNase A, PM1 Buffer should be stored at 4℃.
2) PB Buffer contains chaotropic salt. You should take the appropriate laboratory safety precautions and wear gloves when handling.
3) If there is any precipitate in P2 Buffer (Lysis), incubate on a heating block at 56℃.

Ⅱ DNA Extraction from Plasmid

1) Harvest the 1~5 ml (high-copy number plasmid) or 2~10 ml (low-copy number plasmid) of cultured *E. coli* cells by centrifugation at 6,000 x *g* for 15 min at 4℃ and completely remove the media by pipetting.
2) Add **200 µl** of **PM1 Buffer** to the harvested cell pellet and resuspend by pipetting.
   (Note) PM1 Buffer contains Magnetic Nanobeads, Please shake well the bottle before use.
3) Add **200 µl** of **P2 Buffer** and invert the tube 3~4 times gently.
   (Note) Do not vortex but just invert gently. Vortexing can cause shearing of genomic DNA.
4) Add **200 µl** of **PC Buffer** and invert the tube 3~4 times gently.
   (Note) Again, be cautions not to shear genomic DNA. Genomic DNA and cell debris will form an insoluble complex.
5) Place the tube in ***MagListo*™ -2 Magnetic Separation Rack** with the magnet plate attached and invert the rack gently 3~4 times.
   (Optional) For better yield, centrifuge the tube at >13,000 rpm for 1 min in a micro-centrifuge. Transfer cleared lysate to a new **2 ml (or 1.5 ml)** tube and go step 8.
6) Without removing the tube from *MagListo*™ rack, take the supernatant only and transfer into a new 1.5 ml tube.
7) Repeat the above step 5 and 6 for clearer supernatant.
8) Add **600 µl** of **PB Buffer** and then **50 µl** of **Magnetic NanoBead solution**.
   Invert the tube 3~4 times gently.
   (Note) Please vortex Magnetic NanoBead Solution well before use.
9) Place the tube in ***MagListo*™ -2 Magnetic Separation Rack** with the magnet plate attached and invert the rack gently 3~4 times.
10) Without removing the tube from *MagListo*™ rack, remove the supernatant.
11) Detach the magnet plate from the *MagListo*™ stand. Add **1 ml** of **80% ethanol**.
    Invert the tube until the beads are fully resuspended.
12) Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3~4 times until the beads bind tightly to the magnet.
13) Without removing the tube from *MagListo*™ rack, remove the supernatant.

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14) Detach the magnet plate from the *MagListo*™ stand. Add **1 ml** of **80% ethanol**. Invert the tube until the beads are fully resuspended. Repeat to the above step 12~13.
15) Without removing the tube from *MagListo*™ rack, add **700 µl** of **WE Buffer** to "the opposite side of bead". Close the cap and gently invert the tube twice.
16) Discard the supernatant and completely remove the remaining supernatant by blotting.
17) Detach the magnet plate from the *MagListo*™ rack. Add **100 µl** of **EA Buffer**. Mix thoroughly by vortexing and incubate at 60°C for 1 min.
18) Attach the magnet plate to *MagListo*™ stand and invert the rack gently 3~4 times until the beads bind tightly to the magnet.
19) Without removing the tube from *MagListo*™ rack, transfer the supernatant containing DNA carefully to a sterile microcentrifuge tube.

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

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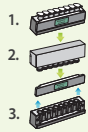
Before You Begin

1) Completely dissolve RNase A powder in PM1 Buffer.
2) If there is any precipitate in P2 Buffer, incubate on a heating block at 56℃.

Step	Image	Description
Resuspension		Add PM1 Buffer to sample and resuspend it completely - Sample : Cell pellet - **PM1 Buffer : 200 µl**
Lysis		Add **P2 Buffer 200 µl** and mix by inverting
Neutralization		Add **PC Buffer 200 µl** and mix by inverting
		Attach magnet and transfer the supernatant into a new 2 ml tube
Binding		Add **PB Buffer 600 µl** and **Magnetic NanoBead 50 µl** and mix until the beads are fully resuspended
		Follow these 3 Magnet Attach/Detach Step 1. Attach magnet 2. Discard the supernatant 3. Detach magnet
1st Wash		Add **80% Ethanol 1 ml** and mix by inverting until the beads are fully resuspended
		Repeat the above magnet attach/detach step (step **1, 2 and 3**)
2nd Wash		Add **80% Ethanol 1 ml** and mix by inverting until the beads are fully resuspended
		Repeat the above magnet attach step (step **1, 2**)
3rd Wash		Add **WE Buffer 700 µl** to "the opposite side of the bead pellet". Close the cap and gently invert the rack twice
		Repeat the above magnet detach step (step **2 and 3**)
Elution		Add **EA Buffer 100 µl** and mix
		60℃ Heating block for 1 min