Protocol

Maxi Plasmid DNA Purification Kit

Product Contents

Cat. No	DNAExt-maxi10	DNAExt-maxi11
Preps	10	25
VP1 Buffer	120ml	265ml
VP2 Buffer	120ml	265ml
VP3 Buffer	120ml	265ml
VPN Buffer	225ml x 2	265ml x 4
VPE Buffer	120ml	265ml
Mini-M Column	20	50
Maxi Ultraflow^{TM} Column	10	25
Protocol	1	1

All buffers should be mixed prior to use

Protocol
Important Notes

1. Briefly centrifuge the RNase A solution tube before use to sediment liquid and apply all of RNase A solution into the VP1 Buffer bottle. Mix well and store at 4℃.
2. If precipitation forms in the VP2 Buffer, incubate at 55℃ for 10 minutes to re-dissolve the salt precipitate. Do not shake the VP2 Buffer; The SDS in the buffer will otherwise foam.
3. Place the VP3 Buffer on ice before use.
4. The volume of VP1-3 Buffer used in the protocol is developed for a100ml sample culture. If the starting sample culture is larger than 100ml, please increase the volume of VP1-3 buffer proportionally.

Procedures

1. Culture plasmid-containing bacterial cells in 100-250ml (for high copy-number plasmids) or 350-500ml (low-copy number plasmids) in LB medium. Grow for 12-16 hours with vigorous shaking at 37°C. Harvest the bacterial cells by centrifugation at 6,000 x g for 15 minutes.
2. Equilibrate Maxi-V500^{TM} Columns by applying 5ml of 98~100%. ethanol. Allow the column to empty by gravity flow and discard the filtrate.
3. Apply 10ml of VPN Buffer to the Maxi-V500^{TM} Column and allow it to flow through by gravity flow and discard the filtrate.
4. Re-suspend the cell pellet in 10ml of VP1 Buffer. The bacterial cells should be completely re-suspended before adding VP2 Buffer.
5. Add 10ml of VP2 Buffer, mix gently by rotating the lysate and let stand for 5 minutes. Do not vortex; vortexing will shear genomic DNA. The lysate should be clear and viscous.
6. Add 10ml of ice-cold VP3 Buffer, mix gently by rotating. After adding VP3 Buffer, a white precipitate should be formed.
7. Centrifuge at 20,000 x g for 15 minutes at 4℃. 20,000 x g corresponds to 12,000 and 13,000 rpm in Beckman JA-17 and Sorvall SS-34 rotors, respectively.
8. Place a Maxi column folded filter in a small funnel, and rinse the filter with 2ml of 70% ethanol, then wash the filter with 5 ml deionized water.
9. Apply the supernatant to the Maxi-V500TM Column and allow it to flow through by gravity flow and discard the filtrate.
10. Wash the column once with 30ml of VPN Buffer by gravity flow and discard the filtrate.
11. Apply 10ml of VPE Buffer to elute DNA by gravity flow.
12. Precipitate DNA by adding 7.5ml (0.75 volumes) of room temperature isopropanol to the eluted DNA. Mix well and centrifuge at 15,000 x g for 30 minutes at 4℃. Carefully remove the supernatant.

13. Wash the DNA pellet with 5 ml of room temperature 70% ethanol and centrifuge at 15,000 x g for 10 minutes. Carefully remove the supernatant.
14. Air-dry the DNA pellet for 10 minutes and dissolve the DNA in 250µl or a suitable volume of TE or ddH_2O.
15. Some insoluble material may also elute out from the column at step 10. To eliminate the insoluble material, load the dissolved DNA sample into a Mini-M™ Column (sitting in a 1.5ml tube) and spin at full speed in a microcentrifuge for 20 seconds, collect the eluted DNA sample in the 1.5 ml tube.
16. Store DNA at -20℃.

Safety, Shipping, Storage

Wear Gloves and eye protection
Shipped at ambient tempeature. Store at ambient temperature up to 24 months
Ground, 2-day and Overnight shipping available.