ACExtract Plasmid Maxi Kit, Endo-free

Cat# NA-P006
store at at 2-8°C

INFORMATION

Size	10T / 20T
Description	Endo-Free Maxi Plasmid Kit uses unique silica membrane technology which can specifically adsorb plasmid DNA efficiently. The whole experimental procedure of plasmid DNA extraction could be finished within 1 h. The following protocol is for the isolation of plasmid DNA from overnight culture of E. coli in LB (Luria-Bertani) medium. The yield and quality of isolated plasmid DNA depend on the cell strain, cell culture condition, lysis of cells, copy number of plasmid, the stability of plasmid and the type of antibiotics. Plasmid DNA prepared by Endo-Free Mini Plasmid Kit is suitable for a variety of routine applications including restriction enzyme digestion, PCR, sequencing, ligation, and transfection to cells
Storage	Endo-free Maxi Plasmid Kit can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, this kit can be stored at 2-8°C. If any precipitate forms in the buffers after storage at 2-8°C, it should be dissolved by warming the buffers at 37°C before use. RNase A (10 mg/ml) can be stored for 12 months at room temperature (15-25°C). After addition of RNase A, Solution I is stable for 12 months at 2-8°C.
Important Notes Before starting	● Add ethanol (96-100%) to Buffer WB before use as the label. ● Add ethanol (96-100%) to DNA Wash Buffer before use as the label. ● Add the provided RNase A solution to Solution I before use, mix, and store at 2-8°C. ● Check all buffer before use for salt precipitation. If necessary, dissolve the buffer by warming at 37°C for several minutes. ● Avoid direct contact of Solution II, immediately close the lid after use. ● All centrifugation steps are carried out at room temperature (15-25°C).
Protocol	10. Pellet up to 100-200 ml bacteria in appropriate vessels by centrifugation at 3,500-5,000 × g for 10 min at room temperature. Decant or aspirate medium and discard. To ensure that all traces of the medium are Page 4 of 12 removed, use a clean paper towel to blot excess liquid from the wall of the vessel. 2. Re-suspend the bacterial pellet in 10 ml Solution I (Ensure that RNase A has been added). The bacteria should be resuspended completely by vortex or pipetting up and down until no cell clumps remain. Note: No cell clumps should be visible after resuspension of the pellet, otherwise incomplete lysis will lower yield and purity. 3. Add 10 ml Solution II and mix gently and thoroughly by inverting the tube 6-8 times. Note: Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min. If the lysate is still not clear, please reduce bacterial pellet. 4. Add 5 ml Buffer NT and mix immediately and gently mix by inverting tube several times until a flocculent white precipitate forms.

Centrifuge at 8,000-12,000 × g for 10 minutes at room temperature to pellet the cellular debris and genomic DNA.

Note: The Buffers must be mixed throughly. If the mixture appears still viscous, brownish and conglobated, more mixing is required to completely neutralize the solution. Complete neutralization of the solution is vital of obtaining good yields.

5. Transfer the cleared lysate to a new 50 ml microcentrifuge tube. Measure the volume of the cleared lysate transferred.
6. Add 0.1 volume Buffer ER. Invert the tube 10 times to mix thoroughly.
7. Incubate on ice for 10 minutes. Invert the tube serval times during the incubation.

Note: After addition of ETR Solution, the lysate should appear turbid, but it should become clear after incubation on ice.

8. Incubate the lysate at 42˚C for 5 minutes. The lysate should appear turbid again.
9. Centrifuge at 4,000 × g for 5 minutes at 25˚C. The ETR Solution will form blue layer at bottom of tube.
10. Transfer the top aqueous phase (cleared lysate) to a new 50 ml tube, add 0.7 volume absolute ethanol (room temperature, 96-100%). Gently invert 6-7 times. Incubate at room temperature for 1-2 minutes.
11. Transfer 20 ml mixture from Step 10 into the GBC Maxi Column. and centrifuge at 4,000 x g for 3 minutes. Discard the flowthrough and put Spin Column back to the collection tube.
12. Repeat Step 11 until all of the mixture has been transferred to the column.
13. Wash the Spin Column by adding 5ml Buffer WB(ensure that ethanol (96%-100%) has been added) and centrifuge at 4,000 x g for 3 minutes. Discard the flowthrough and put Spin Column back to the collection tube.
14. Wash the Spin Column by adding 6ml DNA Wash Buffer(ensure that ethanol (96%-100%) has been added) and centrifuge at 4,000 x g for 3 minutes. Discard the flow-through, and put the Spin Colum back into the Collection Tube.
15. Repeat Step 14.
16. Centrifuge for an additional 2 min at at 4,000 x g for 10 minutes to dry the column matrix..
17. Place column into a clean 50 ml centrifuge tube. Add 2-3 ml (depending on desired concentration of final product) Elution Buffer (or ddH2O) directly onto the column matrix. Allow column to sit 2 min at room temperature. Centrifuge at at 4,000 x g for 5 minutes to elute DNA. This represents approximately 60-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration. Also, preheating the water to 70 C prior to elution may significantly increase yields.
18. Store DNA at -20°C.