ACExtract Plasmid Mini Kit, Endo-free

Cat# NA-P002
store at at 2-8°C

INFORMATION

Size	50T / 200T
Storage	Endo-free Mini 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.
Description	Endo-Free Mini Plasmid Ki 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.
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 12,000 rpm (~13,400× g) in a table-top microcentrifuge at room temperature (15-25°C).
Protocol	1. Harvest 1-5 ml bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm (~13,400 × g) in a conventional, table-top microcentrifuge for 1 min at room temperature (15-25°C), then remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained (For large volume of bacterial cells, please harvest to one tube by several centrifugation step.) 2. Re-suspend the bacterial pellet in 250µl 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 250 µl Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary. Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid

	acidification from CO_2 in the air. 4. Add 125 µl ice cold Buffer NT. Gently invert several times until a flocculent white precipitate forms. 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. Centrifuge at maximum speed (≥13,000 x g) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step. 6. Transfer the cleared lysate to a new 1.5 ml microcentrifuge tube. Measure the volume of the cleared lysate transferred. 7. Add 0.1 volume Buffer ER. Invert the tube 10 times to mix thoroughly. For example: If you transferred 500 µL cleared lysate, then add 50 µl Buffer ER. 8. 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. 9. Incubate the lysate at 42˚C for 5 minutes. The lysate should appear turbid again. 10. centrifuge at 12,000 rpm (~13,400 × g) at room temperature for 3min. The Buffer ER will form blue layer at bottom of tube. 11. Transfer the top aqueous phase (cleared lysate) to a new 1.5 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. 12. Transfer 700 µl mixture from Step 11 into the GBC Column. and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flowthrough and put Spin Column back to the collection tube. 13. Repeat Step 12 until all of the mixture has been transferred to the column. 14. Wash the Spin Column by adding 500 µl Buffer WB(ensure that ethanol (96%-100%) has been added) and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flowthrough and put Spin Column back to the collection tube. 15. Wash the Spin Column by adding 600 µl DNA Wash Buffer(ensure that ethanol (96%-100%) has been added) and centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through, and put the Spin Colum back into the Collection Tube. 16. Repeat Step 15. 17. Centrifuge for an additional 2 min at 12,000 rpm (~13,400 × g) to remove residual DNA Wash Buffer. 18. Place the Spin Column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50-100 µl Elution Buffer to the center of the Spin Column, incubate for 2 min, and centrifuge for 1 min at 12,000 rpm (~13,400 × g). 19. Store DNA at -20°C.