ACExtract Plasmid Mini Kit

Cat# NA-P001
store at at 2-8°C

INFORMATION

Size	50T / 200T
Marker	1kb ladder. 1.0% TAE lipid saccharide swimming.
Storage	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	Extracted from 1-5 ml of bacteria liquid with a high degree of granulated DNA of up to 35ug. On the base of the granules extracted by the lysate method, the column-based nucleic acid technique is combined and is suitable for extracting granulated DNA up to 35 µg high in bacteria cultures from 1 to 5 ml of bacteria. The two different washes wash the column, but also to remove the bacteria containing a variety of substances, applicable to the various requirements of molecular biology.
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 and Solution III, 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 250 µl 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 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 350 µl Solution III and mix immediately and gently by inverting the tube 6-8 times. The solution should become cloudy. Centrifuge for 10 min at 12,000 rpm (~13,400 × g) in a table-top microcentrifuge. Note: To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Solution III. If there is still white precipitation in the supernatant, please centrifuge again. 5. Transfer the supernatant from step 4 to the GBC Column(place in a collection tube) by decanting or pipetting. Centrifuge for 30-60 sec at 12,000 rpm (~13,400 × g). Discard the flow-through and set the Spin Column back into the Collection Tube. 6. 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. 7. 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. 8. Repeat Step 7. 9. Centrifuge for an additional 2 min at 12,000 rpm (~13,400 × g) to remove residual DNA Wash Buffer. 10. Place the Spin Column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50-100 µl ddH2O or TE Buffer to the center of the Spin Column, incubate for 2 min, and centrifuge for 1 min at 12,000 rpm (~13,400 × g).
Extraction of Low Copy or Large Plasmid (>10 kb)	For low copy plasmids and plasmids larger than 10 kb, the amount of bacteria should be increased. It is recommended to use 5-10 ml overnight culture, and the volume of Soluton I、II and III should be increased in proportion. Elution Buffer should be preheated in 65-70°C water bath, and the incubation time for adsorption and elution can be appropriately prolonged to increase the extraction efficiency. The other steps are the same as the above protocol.

PRODUCT USE LIMITATION