ACExtract Plasmid Maxi Kit

Cat# NA-P005
store at Solution I/RNase A at 4 C°

INFORMATION

Size	10T / 20T
Description	Plasmid Mini Kit is based on alkaline lysis technology followed by adsorption of DNA onto silica membrane in the presence of high salt. Phenol extraction and ethanol precipitation are not required. This protocol is designed for purification of plasmid DNA from 100-200ml overnight cultures of E. coli in LB (Luria-Bertani) medium. Yields vary according to plasmid copy number, E.coli strain, and conditions of growth, but 200 ml of overnight culture in LB medium typically produces 500-1200ig of high-copy number plasmid DNA or 50-400 ìg of Low-copy number plasmid. Up to 500 ml culture may be processed when working with low-copy number plasmids. The product is suitable for automated fluorescent DNA sequencing, restriction endonuclease digestion, transfection of mammalian cells, and other manipulations.
Storage	Plasmid isolation components are guaranteed for at least 12 months from the date of purchase when stored as follows: Solution I/RNase A at 4 C, all other material o at 22-25℃.
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 room temperature (15-25°C).
Protocol	1. 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 12ml Solution III and mix immediately and gently mix by inverting tube several times until a flocculent white precipitate forms.

	Centrifuge at 10,000 -12,000× g for 10 minutes at room temperature (preferably at 4℃) to pellet the cellular debris and genomic DNA. 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 Maxi Column (place in a collection tube) by decanting or pipetting. centrifuge at 3,000-5,000 × g for 3-5 min at room temperature to completely pass lysate through column. Discard the flow-through and set the Spin Column back into the Collection Tube. 6. Wash the Spin Column by adding 5mll Buffer WB (ensure that ethanol (96%-100%) has been added) and centrifuge at 3,000-5,000 × g for 3-5 min at room temperature. Discard the flowthrough and put Spin Column back to the collection tube. 7. Wash the Spin Column by adding 6 ml DNA Wash Buffer (ensure that ethanol (96%-100%) has been added) and centrifuge at 3,000-5,000 × g for 3-5 min at room temperature 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 at 4,000 x g for 10 minutes to dry the column matrix. 10. Place column into a clean 50 ml centrifuge tube. Add 2-3 ml (depending on desired concentration of final product) TE buffe(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.