PlasmidPURE™ DNA MINIPREP KIT

ProductInformation

Product No. **P-MINI / P-MINI250**

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TECHNICAL BULLETIN

The PlasmidPURE™ DNA Mini-Prep Kit is a complete plasmid purification system for the quick and inexpensive isolation of high yields of plasmid DNA. The kit utilizes convenient PlasmidPURE spin filters and an optimized buffer system to isolate as much as 15 µg of highly purified plasmid DNA from 1-3 ml of an overnight bacterial culture in minutes. The PlasmidPURE kit does not require the use of phenol, chloroform, cesium chloride, syringes or vacuum manifolds.

Based on a modified alkaline lysis procedure[1], this purification scheme is performed in a few easy steps. First, in the cell lysis step, plasmid DNA is isolated from chromosomal DNA and cell debris. Next, plasmid DNA is bound to the PlasmidPURE spin filter and washed to remove any impurities. Finally, the plasmid DNA is eluted into either a modified TE solution (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), which is included in the kit, or deionized water. The result is highly purified plasmid DNA that is ready for immediate use in restriction and modifying enzyme protocols, and sequencing reactions.

PlasmidPURE Miniprep Kit performs well on both high and low copy number plasmids. Typical results show a consistently high quality plasmid DNA in predominantly supercoiled form. The plasmid can be easily cut with restriction enzymes as demonstrated by analysis on agarose gel electrophoresis. Restriction enzymes can be removed from the plasmid solution using Sigma's NucleiClean™ Nucleic Acid Rapid Isolation Kit (Product No. D-RAP), allowing the linearized plasmid to be ligated with high efficiency. A blue/white selection assay of bacterial colonies has shown that plasmid ends are ligated intact with little or no degradation.

Reagents Provided
Reagents sufficient for 50 or 250 isolations respectively

	P-MINI	P-MINI250
• PlasmidPURE Spin Filters, Product No. F1166	50 each	250 each
• Resuspension Solution A, Product No. R7894	12.5 ml	65 ml
• Lysis Solution B, Product No. L3283	12.5 ml	65 ml
• Neutralization Solution C, Product No. N3029	12.5 ml	65 ml
• 2X Wash Solution D, Product No. W0760	55.0 ml	150 ml
• Elution Buffer E, Product No. E9394	6.0 ml	12.5 ml
• 2.0 ml Microcentrifuge Tubes, Product No. T2676	100 each	500 each

Reagents and Equipment Required but Not Provided

(Sigma product numbers are given where appropriate)

- Microcentrifuge
- Additional 1.5-2.0 ml microcentrifuge tubes (Product No. T9661)
- Ethanol
- Gel loading solution (Product No. G2526)
- Equipment and reagents for agarose gel electrophoresis

Precautions and Disclaimer

Sigma's PlasmidPURE DNA Miniprep Kit is for laboratory use only. Not for drug, household or other uses. Kit contains components which are hazardous. Warning statements are included on the label or in the components section of this bulletin where applicable.

Storage

Store all components at room temperature.

Procedure

Preliminary:

Prepare 1X Wash Solution D adding 55 ml of 95% ethanol for P-MINI (150 ml of 95% ethanol to P-MINI250) to all of 2X Wash Buffer D, for a final volume of 110 ml (or 300 ml).

1. Transfer 1.0-3.0 ml of a 14-16 hour culture of plasmid containing *E. coli* into a microcentrifuge tube and pellet the cells by centrifugation at 5,000-6,000 x g for 30-60 seconds.

2. Carefully remove the supernatant by aspiration or pipetting. Resuspend the cell pellet in 250 µl of resuspension solution A by either vortexing or pipetting up and down until no visible clumps of cells are observed. The final yield of plasmid DNA is directly affected by how effectively the cell pellet is resuspended.

3. Before use, check lysis solution B for SDS precipitation. If necessary, redissolve the SDS by hand warming or incubating at 37°C for a few minutes. Add 250 µl of lysis buffer B to the resuspended pellet and mix gently by inverting the capped tube 10-12 times. The lysed cell suspension should look clear. If it is cloudy, continue mixing until suspension clears.

4. Add 250 µl of neutralization solution C to the lysed cells and mix by gently inverting the capped tube 10-12 times. The solution should coagulate and have a visible, off-white, floating precipitate.

5. Centrifuge at 12,000 x g for 5 minutes to form a firm white pellet along the side or at the bottom of the tube. If a gelatinous mass is present, lysis was incomplete and the plasmid yield will be low.

6. Place a PlasmidPURE spin filter in a 2.0 ml microcentrifuge tube and pour the cleared supernatant directly into the filter and centrifuge at 12,000 x g for 30-60 seconds.

7. Remove the PlasmidPURE spin filter insert from the microcentrifuge tube, pour off the filtrate and replace the filter in the same tube. Add 500 µl of 1X wash solution D and centrifuge at 12,000 x g for 30-60 seconds.

8. Repeat the wash procedure as in step 7 with a two minute centrifugation at 12,000 x g to completely dry the filter of ethanol.

9. Remove the PlasmidPURE spin filter, discard the microcentrifuge tube and place the filter in a fresh tube. Add 50 µl of elution buffer E or deionized water. Incubate at room temperature for 1 minute. Centrifuge at 12,000 x g for 30-60 seconds.

10. Mix 1-5 µl of the DNA with gel loading solution (Product No. G2526) and load on a 1% agarose gel to determine yield and quality.

11. Discard the purification filter and store the eluted DNA at 4°C for immediate use or -20°C for long term storage. For long term storage, store in elution buffer E.

Abbreviated Procedure

1. Centrifuge the cell suspension and decant the supernatant.

2. Resuspend the cells in 250 µl of resuspension solution A.

3. Add 250 µl of lysis solution B, mix gently by inverting. Mix until the suspension clears.

4. Add 250 µl of neutralization solution C, mix gently by inverting.

5. Centrifuge the mixture for 5 minutes.

6. Transfer the supernatant into PlasmidPURE spin filter and centrifuge for 1 minute.

7. Decant the filtrate, add 500 µl of 1X wash solution D and centrifuge for 1 minute.

8. Repeat step 7 with a 2 minute centrifugation.

9. Place the PlasmidPURE spin filter in a fresh receiver tube, add 50 µl of elution buffer E, wait one minute, then centrifuge for 1 minute.

10. Discard the PlasmidPURE spin filter and store the DNA solution at 4°C or -20°C.

Reference

1. Sambrook, J. et. al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Laboratory, Cold Spring Harbor, New York, (1989) p. 1.25.