AddPrep Plasmid Extraction Kit

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Product Code: 10010

Size: 100 preparations

Research Use Only | Store at Room Temp.

Description

AddPrep Plasmid Extraction Kit offer simple, rapid and cost-effective method for isolating plasmid DNA from bacterial cells. This kit is designed for the preparation of up 20 μg of high-purity plasmid DNA from 1 ~ 5 ml overnight E. coli culture in LB medium. Plasmid DNA purified with mini kit is immediately ready for use. Phenol extraction and ethanol precipitation are not required and high-quality plasmid DNA is eluted in a small volume of Elution Buffer.

Kit Components

Solution & Material	Size	Solution & Material	Size
Spin column	100 ea	Optional	32 ml (Add Ethanol 19.2 ml)
Resuspension	30 ml	Washing	15 ml (Add Ethanol 60 ml)
Lysis	30 ml	Elution	20 ml
Neutralization	40 ml	RNase A (10 mg/ml)	0.3 ml

Storage and Stability

AddPrep Plasmid Extraction Kit is stable for 1.5 years when stored in a constant temperature 15 ~ 35℃.

Before You Begin

1. Add RNase A Solution to Resuspension Solution, mix and **store at 4℃ ~ 10℃**.
2. Add ethanol to Optional Solution and Washing Solution before use.
3. Check Lysis Solution and Neutralization Solution for salt precipitation, and salt precipitant can be dissolved by warming at 50℃.

Extraction Protocol

1. Growth of bacterial cultures in tubes or flasks and harvesting: Harvest the bacterial cells from 1 ~ 3ml recombinant E. coli culture by centrifugation of 8,000 rpm in a conventional, table-top micro-centrifuge for 3 min at room temperature.

2. Add 250 μl of Resuspension Solution to the collected cells and completely re-suspend by vortexing or pipetting.

3. Add 250 μl of Lysis Solution and mix by inverting the tube 3~5 times by inverting, gently: Vortexing may cause shearing of genomic DNA. Do not vortex.

4. Add 350 μl of Neutralization Solution and immediately mix by inverting the tube 3~5 times, gently: Genomic DNA and cell debris will be formed and insoluble complex. Do not vortex.

5. Centrifugation the tube at 13,000 rpm, 4 ~ 25℃ for 10 min in micro-centrifuge: A compact white pellet will be appeared at the bottom of the tube.

6. Transfer the supernatant (cleared lysate) to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.

7. (Optional) Add 500 μl of Optional Solution and wait 5 min and centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.

* This step is required if you are using an *endA*+ strains which has a high endonuclease activity. BL21, CJ236, HB101, JM83, JM 101, JM110, LE392, NM series strains, PR series strains, Q358, PR1, TB1, TG1, Y10 series strains, BMH71-18 and ES1301 are *end*A+ strains, thus they produce highly active endonucleases that can degrade plasmids. Denaturation step is not required for the DH5 , XL1-Blue, BJ5183, DH1, DH20, DH21, JM103, JM105, JM106, JM107, JM108, JM109, MM294, SK1590, SK1592, SK2267, SRB and XLO strains.

8. Add 700 μl of Washing Solution to the spin column with collection tube and centrifuge at 13,000 rpm for 1 min: Pour off the flow-through and assemble the spin column with the 2.0 ml collection tube.

9. Dry the spin column by additional centrifugation at 13,000 rpm for 1 min to remove the residual ethanol in spin column.

10. Transfer the spin column to the new 1.5 ml micro-centrifuge tube (Not provided).

11. Add 50 ~ 100 μl of Elution Solution to the spin column with micro-centrifuge tube, and let stand for at least 1 min.

12. Elute the plasmid DNA by centrifugation at 13,000 rpm for 1 min.