Wizard®PlusSV Minipreps DNA Purification System

Introduction

The Wizard® *Plus* SV Minipreps DNA Purification System provides a simple and reliable method for rapid isolation of plasmid DNA. The entire miniprep procedure can be completed in 30 minutes or less, depending on the number of samples processed. This system can be used to isolate any plasmid from *E.coli* hosts but works most efficiently when the plasmid is <20,000bp in size. Plasmid DNA can be purified from 1–10ml of overnight cultures of *E. coli* with the Wizard® *Plus* SV Minipreps DNA Purification System. The yield of plasmid will vary depending on a number of factors, including the volume of bacterial culture, plasmid copy number, type of culture medium and bacterial strain used.

Materials

- temporary 1.5ml microcentifuge tube for pellet
- Spin Column and Collection Tube
- 1.5ml microcentifuge tube for final storage
- 1–10ml of overnight culture
- Cell Resuspension Solution
- Cell Lysis Solution
- Alkaline Protease Solution
- Neutralization Solution
- Wash Solution (ethanol added)
- Nuclease-Free Water
- centrifuge

Procedure

Centrifugation Protocol

1. Pellet 1–10ml of overnight culture for 5 minutes.
2. Thoroughly resuspend pellet with 250µl of Cell Resuspension Solution.
3. Add 250µl of Cell Lysis Solution to each sample; invert 4 times to mix.
4. Add 10µl of Alkaline Protease Solution; invert 4 times to mix. Incubate 5 minutes at room temperature.
5. Add 350µl of Neutralization Solution; invert 4 times to mix.
6. Centrifuge at top speed for 10 minutes at room temperature.

Binding of Plasmid DNA

7. Insert Spin Column into Collection Tube.
8. Decant cleared lysate into Spin Column.
9. Centrifuge at top speed for 1 minute at room temperature. Discard flowthrough, and reinsert Column into Collection Tube.

Washing

10. Add 750µl of Wash Solution (ethanol added). Centrifuge at top speed for 1 minute. Discard flowthrough and reinsert column into Collection Tube.
11. Repeat Step 10 with 250µl of Wash Solution.
12. Centrifuge at top speed for 2 minutes at room temperature

Elution

13. Transfer Spin Column to a sterile 1.5ml microcentrifuge tube, being careful notto transfer any of the Column Wash Solution with the Spin Column. If the SpinColumn has Column Wash Solution associated with it, centrifuge again for 1 minute at top speed, then transfer the Spin Column to a new, sterile 1.5mlmicrocentrifuge tube.
14. Add 100µl of Nuclease-Free Water to the Spin Column. Centrifuge at topspeed for 1 minute at room temperature.
15. Discard column, and store DNA at –20°C or below.