GenElute™ Plasmid Miniprep Kit

Introduction

Purpose to extract plasmids from cells using the GenElute™ Plasmid Miniprep Kit.

Materials

- Resuspension Solution
- RNase A solution
- Lysis Solution
- Neutralisation/Binding Solution
- Column Preparation Solution
- Wash Solution Concentrate
- Elution Solution Concentrate
- Elution Solution (10 mM Tris-HCL 1 mM EDTA, pH approx. 8.0)
- GenElute Miniprep Binding Columns
- 2 mL collection Tubes

Procedure

Preparation in case of opening a new kit

1. Thoroughly mix reagents (and check that there is no precipitation)
2. Resuspension of solution. Spin the tube of the RNase A Solution briefly to collect the solution in the bottom of the tube. Add 13 µL (for 10 prep package), 78 µL (for 70 prep package) or 500 μL (for 350 prep package) of the RNase A Solution to the Resuspension Solution prior to initial use. Store at 4 °C.
3. Wash Solution. Dilute the Wash Solution Concentrate with 10 mL (10 prep package), 100 mL (70 prep package), or 300 mL (350 prep package) of 95–100% ethanol prior to initial use. After each use, tightly cap the diluted wash solution to prevent the evaporation of ethanol

Main procedure

1. Harvest cells

   Pellet 5 mL of an overnight recombinant *E. coli* culture by centrifugation. Transfer the appropriate volume of the recombinant *E. coli* culture to a microcentrifuge tube and pellet cells at ≥12,000 x g for 1 minute. Discard the supernatant.

2. Resuspend Cells

   Completely resuspend the bacterial pellet with 200 µL of the Resuspension Solution. Vortex or pipette up and down to thoroughly resuspend the cells until homogeneous.

3. Lyse cells

   Lyse the resuspended cells by adding 200 µL of the Lysis Solution. Immediately mix the contents by gentle inversion (6–8 times) until the mixture becomes clear and viscous. Do not vortex. Do not allow the lysis reaction to exceed 5 minutes.

4. Neutralize

   Precipitate the cell debris by adding 350 µL of the Neutralization/Binding Solution. Gently invert the tube 4–6 times. Pellet the cell debris by centrifuging at ≥12,000 x g or maximum speed for 10 minutes. A cloudy, viscous precipitate should form. If the supernatant contains a large amount of floating particles after centrifugation, re-centrifuge the supernatant.

5. Prepare Column

   Insert a GenElute Miniprep Binding Column into a provided microcentrifuge tube, if not already assembled. Add 500 µL of the Column Preparation Solution to each miniprep column and centrifuge at ≥12,000 x g for 30 seconds to 1 minute. Discard the flow-through liquid.

6. Load cleared lysate

   Transfer the cleared lysate from step 7 to the column prepared in step 8 and centrifuge at ≥12,000 x g for 30 seconds to 1 minute. Discard the flow-through liquid.

7. Optional wash

   Add 500 µL of the Optional Wash Solution to the column. Centrifuge at ≥12,000 x g for 30 seconds to 1 minute. Discard the flow-through liquid. Note: When working with bacterial strains containing the wild-type EndA+ gene, such as HB101, JM101, and the NM and PR series, the Optional Wash step is necessary to avoid nuclease contamination of the final plasmid DNA product

8. Wash column

   Add 750 µL of the diluted Wash Solution to the column. Centrifuge at ≥12,000 x g for 30 seconds to 1 minute. Discard the flow-through liquid and centrifuge again at maximum speed for 1 to 2 minutes without any additional Wash Solution to remove excess ethanol.

9. Elute DNA

   Transfer the column to a fresh collection tube. Add 100 µL of Elution Solution or molecular biology reagent water to the column. For DNA sequencing and other enzymatic applications, use water or 5 mM Tris-HCl, pH 8.0, as an eluant. Centrifuge at ≥12,000 x g for 1 minute. The DNA is now present in the eluate and is ready for immediate use or storage at –20 °C. Note: If a more concentrated plasmid DNA preparation is required, the elution volume may be reduced to a minimum of 50 µL. However, this may result in a reduction in the total plasmid DNA yield.