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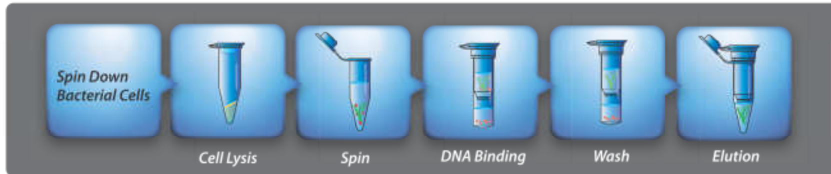

HiYield™ Plasmid Midi Kit Protocol Book

High Yield Plasmid DNA from 50-100 ml of Bacterial Cultures

Cat. No. **YPDI25**

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HiYield™ Plasmid Midi Kit

Cat. No. **YPDI25**
25 midi preps / kit
PD1 Buffer: 110 ml
PD2 Buffer: 110 ml
PD3 Buffer: 160 ml
W1 Buffer : 220 ml
Wash Buffer (concentrated): 100 ml *
Elution Buffer: 60 ml
RNase A (50 ug/ul): 400 µl
PDI Column: 25 pcs

Sample: 50-100 ml of Bacterial Cultures
Yield: Low-Copy Plasmid up to 250 µg / High-Copy Plasmid up to 500 µg
Format: Spin Columns
Operation Time: 30~40 Minutes

Add provided RNase A to PD1 Buffer and store at 4°C.
If precipitates have formed in PD2 Buffer, warm the buffer in a 37°C waterbath, followed by gentle shaking to dissolve.
* Add 200 ml ethanol(96-100%) to 100 ml of Wash Buffer prior to the initial use.

Description

HiYield™ Plasmid Midi Kit is designed for rapid isolation of up to 500 µg plasmid or cosmid DNA from 50-100 ml of bacterial cultures without phenol extraction and alcohol precipitation. Typical yields are 200-350 µg for high-copy plasmid or 30-100 µg for low-copy plasmid from 50 ml of bacterial cultures. The entire procedure can be completed within 40 minutes. The purified plasmid DNA is ready for use in Restriction Enzyme Digestion, Ligation, PCR, and Sequencing Reactions.

Features

High, reproducible recovery of plasmid DNA.
Rapid and simple procedure.
No phenol, chloroform or alcohol.

Applications

Purified DNA is ready for direct use in DNA Sequencing, DNA Library Screening and Analysis, Restriction Digestion, Ligation and Transformation.

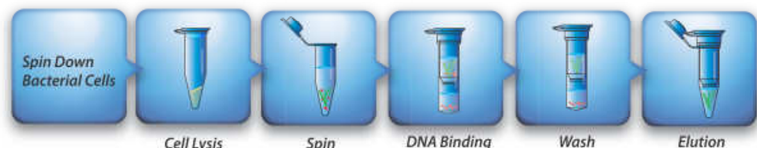
Quality Control

The quality of HiYield™ Plasmid Midi Kits are tested on a lot-to-lot basis by isolation of plasmid DNA from 50 ml overnight culture of E.coli DH5a transformed with the plasmid pBluescript (A_{600} > 2 units/ml). Purified DNA is quantified with a spectrophotometer and the yield of plasmid DNA is more than 200 µg with A_{260}/A_{280} ratio 1.7 to 1.9. The purified DNA is used in Eco R1 digestion and checked by electrophoresis.

Note: (1) For research use only. Not for use in diagnostic or therapeutic procedures.
(2) PD3 Buffer and W1 Buffer contain guanidine hydrochloride which is harmful and irritant agent. During Operation, always wear a lab coat, disposable gloves and protective goggles.

Reference: (1) Birnboim, H. C., and Doly, J. (1997) Nucleic Acids Res. 7, 1513.
(2) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Protocol



Additional requirements:

* 96% ~ 100% Ethanol.
* Sterile, DNase-free pipette tips and centrifuge tubes.

Things to do before starting:

* Add provided RNase A to PD1 Buffer and store at 4°C.
* If precipitates have formed in PD2 Buffer, warm the buffer in a 37°C waterbath, followed by gentle shaking to dissolve.
* Add absolute ethanol to the Wash Buffer prior to the initial use (see the bottle label for details).

Harvesting

1. Transfer 50 ml of bacterial culture to a 50 ml centrifuge tube (not provided).
2. Centrifuge for 5 minutes at full speed (approx. 13,000 rpm) and discard the supernatant.
(If more than 50 ml of bacterial culture is used, repeat the Harvesting Step.)

Resuspension

3. Add 4 ml of PD1 Buffer (RNase A added) to the tube and resuspend the cell pellet by vortexing or pipetting.

Lysis

4. Add 4 ml of PD2 Buffer and mix gently by inverting the tube 10 times. Do not vortex to avoid shearing genomic DNA.
5. Let stand for 2 minutes at room temperature until lysate clears.

Neutralization

6. Add 6 ml of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex.
7. Centrifuge for 10 minutes at full speed (approx.13,000 rpm).

DNA Binding

8. Place a PDI Column in a 50 ml centrifuge tube.
9. Apply the clear lysate (supernatant) from Step 7 to the PDI Column. Be sure the lid of 50 ml centrifuge tube is twisted tightly.
10. Centrifuge at full speed (approx. 13,000 rpm) for 3 minutes.
11. Discard the flow-through and place the PDI Column back into the 50 ml centrifuge tube.

Wash

12. Add 8 ml of W1 Buffer in the PDI Column. Be sure the lid of 50 ml centrifuge tube is twisted tightly.
13. Centrifuge at full speed (approx. 13,000 rpm) for 3 minutes.
14. Discard the flow-through and place the PDI Column back into the 50 ml centrifuge tube.
15. Add 12 ml of Wash Buffer (ethanol added) in the PDI Column. Be sure the lid of 50 ml centrifuge tube is twisted tightly.
16. Centrifuge at full speed (approx. 13,000 rpm) for 3 minutes.
17. Discard the flow-through and place the PDI Column back into the 50 ml centrifuge tube.
18. Centrifuge again for 3 minutes at full speed to dry the column matrix.

DNA Elution

19. Transfer the dried PDI Column to a new 50 ml centrifuge tube (Not provided).
20. Add 2 ml of Elution Buffer or distilled water into the center of the column matrix. Avoid residual buffer adhering to the wall of the column.
21. Let stand for 2 minutes until Elution Buffer or distilled water is absorbed by the matrix.
22. Centrifuge for 2 minutes at full speed to elute purified DNA.

Troubleshooting

Problem	Possible Reasons/ Solution
Low yield	**Bacterial cells were not lysed completely** Too many bacterial cells were used. If more than 10 A_{600} units of bacterial cultures are used, separate them into multiple tubes. Following PD 3 Buffer addition, break up the precipitate by inverting or pipetting to ensure higher yield.
	Incorrect Wash Buffer Ensure Ethanol was added to Wash Buffer prior to use .
	Incorrect DNA Elution Step Ensure Elution Buffer was added into the center of PDI Column matrix and was completely absorbed.
	Incomplete DNA Elution If plasmid DNA is larger than 10 kb, use preheated Elution Buffer (60-70^{0}C) in Elution Step to improve the elution efficiency.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** After wash step, dry PDI Column with additional centrifugation at top speed for 5 minutes or incubation at 60^{0}C for 5 minutes.
	RNA contamination Prior to using PD1 Buffer, ensure that RNase A was added. If RNase A added PD1 Buffer is out of date, add additional RNase A. Too many bacterial cells were used, reduce sample volume.
	Genomic DNA contamination Do not use overgrown bacterial culture. During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing.
	Nuclease contamination If host cells have high nuclease activity (e.g., endA$^+$ strains), perform this Optional Wash Step to remove residual nuclease. After DNA Binding Step, add 8 ml of W1 Buffer into PDI Column and incubate for 2 minutes at room temperature. Centrifuge at 6000 x g (8,000 rpm) for 5 minutes. Continue with standard Wash Step.

HiYield™ Plasmid Midi Kit

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