96-Well Plasmid Kit

For research use only

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Sample : 1-2 ml of cultured bacterial cells
Binding Capacity : 10 μg/well
Operation : centrifuge/vacuum manifold
Yield : 5-10 μg for high-copy plasmid
0.5-5 μg for low-copy plasmid
Operation time : within 60 minutes
Application : Fluorescent/Radioactive Sequencing, Restriction Enzyme Digestion, Library Screening, Ligation, Transformation and PCR

Introduction

The 96-Well Plasmid Kit was designed for rapid isolation of plasmid DNA from 1-2 ml of cultured bacterial cells. Modified Alkaline Lysis method (1) and RNase A treatment are used to obtain clear cell lysate with minimal genomic DNA/RNA contaminants. In the presence of chaotropic salt, plasmid DNA in the lysate binds to the glass fiber matrix (2) of each well of the plate. Contaminants are removed with a Wash Buffer (containing ethanol) and the purified plasmid DNA is eluted by a low salt Elution Buffer or TE. Typical yields are 5-10 μg for high-copy number plasmid or 0.5-5 μg for low-copy number plasmid. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 1 hour. The purified plasmid DNA is ready for use in various downstream applications.

Quality Control

The quality of the 96-Well Plasmid Kit is tested on a lot-to-lot basis by isolating plasmid DNA from a 1.5 ml overnight *E. coli* (DH5α) culture, containing plasmid pBluescript (A600 > 2 U/ml). More than 8 μg of plasmid DNA can be quantified with a spectrophotometer. The purified plasmid (1 μg) is used in EcoR I digestion, and checked by electrophoresis.

Kit Contents

Name	PDA02	PDA04	PDA10
PD 1 Buffer*	25 ml	65 ml	65 ml x 2
PD 2 Buffer**	25 ml	75 ml	75 ml x 2
PD 3 Buffer	45 ml	100 ml	100 ml x 2
W1 Buffer	60 ml	130 ml	130 ml x 2
Wash Buffer*** (Add Ethanol)	12.5 ml (50 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer (10 mM Tris-HCl, pH 8.5 at 25ºC)	30 ml	60 ml	120 ml
RNase A (50 mg/ml)	50 μl	130 μl	130 μl x 2
Plasmid Plate	2 pcs	4 pcs	10 pcs
0.35 ml Collection Plate	2 pcs	4 pcs	10 pcs
Adhesive Film	4 pcs	8 pcs	20 pcs

Order Information

Product Name	Package Size	Cat. No.
High-Speed Plasmid Mini Kit	100/300 preps	PD100/300
High-Speed Plasmid Mini Kit (10-50 Kb)	100/300 preps	PDL100/300
High-Speed Plasmid Advance Kit (50-100 ml bacterial culture)	25 preps	PA025
Geneaid Plasmid Midi Kit	25 preps	PI025
Geneaid Plasmid Midi Kit (Endotoxin Free)	25 preps	PIE25
Geneaid Plasmid Maxi Kit	10/25 preps	PM010/025
Geneaid Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	PME10/E25
96-Well Plasmid Kit	2/4/10 x 96 Wells	PDA02/04/10
Vacuum Manifold (Accessories)	1 Set	ZVF01

*Add provided RNase A to the PD1 Buffer and store at 4ºC
**If precipitates have formed in the PD2 Buffer, warm the buffer in a 37ºC water bath to dissolve
***Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use

Caution

PD3 Buffer and W1 Buffer contain guanidine hydrochloride which is a harmful irritant. During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

References

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

96-Well Plasmid Kit Centrifuge Protocol

➢ Additional requirements: centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol

Step	Procedure
Step1 Harvesting	• Transfer 1.5 ml of cultured bacterial cells to a 2 ml collection plate and centrifuge for 10 minutes at 3,000 x g. • Remove the culture medium.
Step 2 Re-suspension	• Add **100 μl of PD1 Buffer** (RNase A added) to each well of the plate and re-suspend the cell pellet by pipetting or vortex.
Step 3 Lysis	• Add **100 μl of PD2 Buffer** to each well and mix gently by shaking. • Let stand at room temperature for at least 2 minutes to ensure the lysate is clear.
Step 4 Neutralization	• Add **150 μl of PD3 Buffer** and mix immediately by shaking gently, then centrifuge for 10 minutes at 3,000 x g. Following centrifugation, a denatured protein pellet and clear lysate (supernatant) will be present in each well.
Step 5 DNA Binding	• Place a **Plasmid Plate** on a new 2 ml collection plate. • Carefully transfer ONLY the clear lysate (supernatant) from Step 4 to the **Plasmid Plate**. • Centrifuge for 5 minutes at 3,000 x g. • Discard the flow-through and place the **Plasmid Plate** back on the 2 ml collection plate.
Step 6 Wash	• Add **250 μl of W1 Buffer** into each well of the **Plasmid Plate** and centrifuge for 5 minutes at 3,000 x g. • Add **250 μl of Wash Buffer** (ethanol added) into each well of the **Plasmid Plate** to wash again and centrifuge for 5 minutes at 3,000 x g. • Discard the flow-through and place the **Plasmid Plate** back on the 2 ml collection plate. • Centrifuge for 10 minutes at 3,000 x g to remove any ethanol residue.
Step 7 DNA Elution	• Transfer the **Plasmid Plate** to a **0.35 ml Collection Plate**. • Add **50-100 μl of Elution Buffer** or TE into the center of each membrane matrix. • Let stand for at least 2 minutes to ensure the **Elution Buffer** or TE is absorbed. • Centrifuge for 5 minutes at 3,000 x g to elute the purified DNA.

96-Well Plasmid Kit Vacuum Protocol

➢ Additional requirements: centrifuge system for 96-well plates, multi-well plate vacuum manifold, 2 ml collection plate, absolute ethanol

Step	Procedure
Step1 Harvesting	• Transfer 1.5 ml of cultured bacterial cells to a 2 ml collection plate and centrifuge for 10 minutes at 3,000 x g. • Remove the supernatant.
Step 2 Re-suspension	• Add **100 μl of PD1 Buffer** (RNase A added) to each well of the plate and re-suspend the cell pellet by pipetting or vortex.
Step 3 Lysis	• Add **100 μl of PD2 Buffer** and mix gently by shaking. • Let stand at room temperature for at least 2 minutes to ensure the lysate is clear.
Step 4 Neutralization	• Add **150 μl of PD3 Buffer** and mix immediately by shaking gently and centrifuge for 10 minutes at 3,000 x g. Following centrifugation, a denatured protein pellet and clear lysate (supernatant) will be present in each well.
Step 5 DNA Binding	• Place a 2 ml collection plate on the base of the vacuum manifold and place a **Plasmid Plate** on top. • Carefully transfer ONLY the clear lysate (supernatant) from Step 4 to the **Plasmid Plate** (approximately 350 μl). • Apply vacuum at 10 inches Hg for at least 5 minutes to ensure the wells have emptied.
Step 6 Wash	• Turn off the vacuum pump and add **250 μl of W1 Buffer** to each well of the **Plasmid Plate**. • Apply vacuum at 10 inches Hg for at least 5 minutes to ensure the wells have emptied. • Turn off the vacuum pump and add **250 μl of Wash Buffer** (ethanol added) to each well of the **Plasmid Plate**. • Apply vacuum at 10 inches Hg for at least 5 minutes to ensure the wells have emptied. • Apply vacuum for an additional 10 minutes (or incubate at 60ºC for 5-10 minutes) to remove any ethanol residue.
Step 7 DNA Elution	• Turn off the vacuum pump and transfer the **Plasmid Plate** to a **0.35 ml Collection Plate**. • Add **50-100 μl of Elution Buffer** or TE into the center of each membrane matrix. • Let stand for at least 2 minutes to ensure the **Elution Buffer** or TE is absorbed. • Centrifuge for 5 minutes at 3,000 x g to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	**Bacterial cells were not lysed completely** • If more than 10 OD_{600} units of bacterial culture are used, dilute into multiple tubes. • Following PD3 Buffer addition, pipetting or gentle shaking will help to ensure the sample is homologous. **Incorrect DNA Elution Step** • Ensure that Elution Buffer is added into the center of each well. **Incomplete DNA Elution** • If plasmid DNA are larger than 10 Kb, use pre-heated Elution Buffer (60~70ºC) in the Elution step.
Eluted DNA does not perform well in downstream applications	**Residual Ethanol contamination** • Following Wash step, dry the Plasmid Plate with extended vacuum/centrifugation time of approximately 5 minutes. **RNA contamination** • Prior to using PD1 Buffer, ensure RNase A is added. **Genomic DNA contamination** • Do not use overgrown bacterial cultures. • During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing.