

96-Well Plasmid Kit

For research use only

Sample	: 1-2 ml of cultured bacterial cells
Binding Capacity	: 10 µg/well
Format	: 96-well plates
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

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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	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 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 4°C.

**If precipitates have formed in the PD2 Buffer, warm the buffer in a 37°C water bath to dissolve.

***Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

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

- Add provided RNase A to the PD1 Buffer and store at 4°C.
- If precipitates have formed in the PD2 Buffer, warm the buffer in a 37°C water bath to dissolve.
- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol

Step 1 Harvesting	<ul style="list-style-type: none"> ● Transfer 1.5 ml of cultured bacterial cells to a 2 ml collection plate. ● Centrifuge for 10 minutes at 3,000 x g. ● Remove the medium.
Step 2 Resuspension	<ul style="list-style-type: none"> ● Add 100 µl of PD1 Buffer (RNase A added) to each well of the plate and resuspend the cell pellet by pipetting or vortex.
Step 3 Lysis	<ul style="list-style-type: none"> ● Add 100 µl of PD2 Buffer to each well and mix gently by shaking. ● Let stand at room temperature for 2 minutes or until the lysate clears.
Step 4 Neutralization	<ul style="list-style-type: none"> ● Add 150 µl of PD3 Buffer and mix immediately by shaking gently. ● 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	<ul style="list-style-type: none"> ● 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	<ul style="list-style-type: none"> ● Add 250 µl of W1 Buffer into each well of the Plasmid Plate. ● 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	<ul style="list-style-type: none"> ● 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 2 minutes or until 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

- Add provided RNase A to the PD1 Buffer and store at 4°C.
- If precipitates have formed in the PD2 Buffer, warm the buffer in a 37°C water bath to dissolve.
- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional requirements: centrifugation system for 96-well plates, multi-well plate vacuum manifold, 2 ml collection plate, absolute ethanol

Step 1 Harvesting	<ul style="list-style-type: none"> ● Transfer 1.5 ml of cultured bacterial cells to a 2 ml collection plate. ● Centrifuge for 10 minutes at 3,000 x g. ● Remove the supernatant.
Step 2 Re-suspension	<ul style="list-style-type: none"> ● Add 100 µl of PD1 Buffer (RNase A added) to each well of the plate and resuspend the cell pellet by pipetting or vortex.
Step 3 Lysis	<ul style="list-style-type: none"> ● Add 100 µl of PD2 Buffer and mix gently by shaking. ● Let stand at room temperature for 2 minutes or until the lysate clears.
Step 4 Neutralization	<ul style="list-style-type: none"> ● Add 150 µl of PD3 Buffer and mix immediately by shaking gently. ● 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	<ul style="list-style-type: none"> ● Place a 2 ml collection plate on the base of the vacuum manifold and place a Plasmid Plate on top of the vacuum manifold. ● Carefully transfer ONLY the clear lysate (supernatant) from Step 4 to the Plasmid Plate (approximately 350 µl). ● Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied.
Step 6 Wash	<ul style="list-style-type: none"> ● 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 5 minutes or until 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 to wash again. ● Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied. ● Apply vacuum for an additional 10 minutes (or incubate at 60°C for 5-10 minutes) to remove any ethanol residue.
Step 7 DNA Elution	<ul style="list-style-type: none"> ● 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 2 minutes or until 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 <ul style="list-style-type: none"> ● If more than 10 OD₆₀₀ 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 <ul style="list-style-type: none"> ● Ensure that Elution Buffer is added into the center of each well.
	Incomplete DNA Elution <ul style="list-style-type: none"> ● If plasmid DNA are larger than 10 Kb, use preheated Elution Buffer (60~70°C) in the Elution step.
Eluted DNA does not perform well in downstream applications	Residual ethanol contamination <ul style="list-style-type: none"> ● Following the Wash step, dry the Plasmid plate with extended vacuum or centrifugation time of approximately 5 minutes.
	RNA contamination <ul style="list-style-type: none"> ● Prior to using PD1 Buffer, ensure RNase A is added.
	Genomic DNA contamination <ul style="list-style-type: none"> ● Do not use overgrown bacterial cultures. ● During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing.