Large Plasmid DNA Extraction Kit

For research use only

Sample: 1-4 ml of cultured bacterial cells

Binding Capacity: up to 30 µg of 10-50 kb plasmid DNA

Format: spin column

Operation Time: within 15 minutes

Elution Volume: 50-100 µl

Storage: dry at room temperature (15-25ºC)

Geneaid

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

The Large Plasmid DNA Extraction Kit was designed for rapid isolation of 10-50 kb plasmid or cosmid DNA from 1-4 ml of cultured bacterial cells. A modified alkaline lysis method and RNase treatment are used to obtain clear cell lysate with minimal genomic DNA and RNA contaminants. In the presence of chaotropic salt, plasmid DNA in the lysate binds to the glass fiber matrix of the spin column. 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 20-30 µg for high-copy number plasmid or 3-10 µg for low-copy number plasmid from 4 ml of cultured bacterial cells. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 15 minutes. The purified plasmid DNA is ready for use in Restriction Enzyme Digestion, Ligation, PCR, and sequencing reactions.

Quality Control

The quality of the Large Plasmid DNA Extraction Kit is tested on a lot-to-lot basis by isolating plasmid DNA from a 4 ml overnight *E. coli* (DH10β) culture, containing plasmid pBluescript (A600 > 2 U/ml). Following the purification process, a yield of more than 20 µg is obtained and the ratio of A260/A280 is between 1.7-1.9. The purified plasmid (1 µg) is used in *Eco*RI digestion and analyzed by electrophoresis.

Kit Contents

Component	PDL004	PDL100	PDL300
PDL1 Buffer*	1 ml	25 ml	65 ml
PDL2 Buffer**	1 ml	25 ml	75 ml
PDL3 Buffer	1.5 ml	45 ml	100 ml
WL1 Buffer	2 ml	45 ml	130 ml
Wash Buffer*** (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	6 ml	30 ml
RNase A (50 mg/ml)	Added	50 µl	130 µl
LP Columns	4	100	300
2 ml Collection Tubes	4	100	300

Order Information

Plasmid DNA Purification		
Product	Package Size	Catalogue Number
Presto™ Mini Plasmid Kit	100/300 preps	PDH100/300
Presto™ Midi Plasmid Kit	25 preps	PIF025
Presto™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIFE25
Large Plasmid DNA Extraction Kit	100/300 preps	PDL100/300
High-Speed Plasmid Advance Kit (50-100 ml)	25 preps	PA025
Geneaid™ Midi Plasmid Kit	25 preps	PI025
Geneaid™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIE25
Presto™ Plasmid DNA Concentration Kit	250/500/1000 preps	PC0250/500/1000
Geneaid™ Maxi Plasmid Kit	10/25 preps	PM010/25
Geneaid™ Maxi Plasmid Kit (Endotoxin Free)	10/25 preps	PME10/25
Presto™ 96 Well Plasmid Kit	4/10 x 96 preps	96PDV04/10, 96PDC04/10

*For PDL100 and PDL300 add provided RNase A to PDL1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Store the PDL1 and RNase A mixture at 2-8ºC for up to 6 months. For PDL004 samples, RNase A was already added to PDL1.
**If precipitates have formed in PDL2 Buffer, warm in a 37ºC water bath, followed by gentle shaking to dissolve.
***Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use.

Caution

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Large Plasmid DNA Extraction Kit Protocol

IMPORTANT BEFORE USE!

1. For PDL100 and PDL300 add provided RNase A to PDL1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. Store the PDL1 and RNase A mixture at 2-8ºC for up to 6 months. For PDL004 samples, RNase A was already added to PDL1.
2. If precipitates have formed in PDL2 Buffer, warm in a 37ºC water bath, followed by gentle shaking to dissolve.
3. Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use.

Additional requirements: microcentrifuge tubes, absolute ethanol

Harvesting	• Transfer **1.5 ml of cultured bacterial cells** to a 1.5 ml microcentrifuge tube. • Centrifuge at 14-16,000 x g for 1 minute then discard the supernatant. • If more than 1.5 ml of cultured bacterial cells is used, repeat the Harvesting step using the same 1.5 ml tube.
Step 1 Resuspension	• Add **200 µl of PDL1 Buffer (make sure RNase A was added)**. • Resuspend the cell pellet by vortex or pipetting.
Step 2 Lysis	• Add **200 µl of PDL2 Buffer** then mix gently by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. • Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. At this time, pre-heat the required **Elution Buffer** to 70ºC (for Step 6 DNA Elution).
Step 3 Neutralization	• Add **300 µl of PDL3 Buffer** and mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. • Centrifuge at 14-16,000 x g for 3 minutes.
Step 4 DNA Binding	• Place a **LP Column** in a **2 ml Collection Tube**. • Transfer **the supernatant** from Step 3 to the **LP Column** then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the **LP Column** back in the **2 ml Collection Tube**.
Step 5 Wash	• Add **400 µl of WL1 Buffer** into the **LP Column** then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the **LP Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **LP Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow through then place the **LP Column** back in the **2 ml Collection Tube**. • Centrifuge at 14-16,000 x g again for 3 minutes to dry the column matrix.
Step 6 DNA Elution	• Transfer the dry **LP Column** to a new 1.5 ml microcentrifuge tube. • Add **50 µl of pre-heated Elution Buffer** or TE into the center of the column matrix. • Let stand for at least 2 minutes to ensure the **Elution Buffer** or TE is absorbed by the matrix. • Centrifuge at 14-16,000 x g for 2 minutes to elute the purified plasmid 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 PDL3 Buffer addition, pipetting or inverting will help to ensure the sample is homogeneous.
	Incorrect DNA Elution Step • Ensure that Elution Buffer is added into the center of the LP Column matrix and is completely absorbed.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** • Following the Wash step, dry the LP Column with additional centrifugation at 14-16,000 x g for 5 minutes.
	RNA contamination • Prior to using PDL1 Buffer, be sure RNase A is added.
	Genomic DNA contamination • Do not use overgrown bacterial cultures. • During PDL2 and PDL3 Buffer addition, mix gently to prevent genomic DNA shearing.
	Nuclease contamination • Following the DNA Binding step, add 400 µl of WL1 Buffer into the LP Column and incubate for 2 minutes at room temperature. Centrifuge the LP Column at 14-16,000 x g for 30 seconds and proceed with the standard wash step.