Plasmid DNA Miniprep Kit

For research use only

Cat No. DMDPN100 & DMDPN300

Sample: up to 4 ml bacterial cells

Yield: up to 30μg of plasmid

Introduction

The BioDiamond Plasmid DNA Miniprep Kit provides a fast, simple, and cost-effective spin-column method for isolation of plasmid DNA from cultured bacterial cells. The BioDiamond Plasmid DNA Miniprep Kit is based on alkaline lysis of bacterial cells followed by binding of DNA onto the glass fiber matrix of the spin column in the presence of a high amount of salt. Phenol extraction and ethanol precipitation are not required, and high-quality plasmid DNA is eluted with a small volume of Tris buffer (included in each kit) or water (pH is between 7.0 and 8.5). Plasmid DNA purified with the BioDiamond Plasmid DNA Miniprep Kit is suitable for a variety of routine applications including restriction enzyme digestion, sequencing, library screening, in vitro translation, transfection of robust cells, ligation and transformation. The entire procedure can be completed within 20 minutes.

Kit Contents

Catalog No.	DMDPN100	DMDPN300
S1 Buffer	25 ml	65 ml
S2 Buffer	25 ml	65 ml
S3 Buffer	35 ml	95 ml
W1 Buffer	45 ml	125 ml
W2 Buffer (Add Ethanol)	15 ml (60 ml)	25 ml × 2 (100 ml × 2)
EL Buffer	10 ml	30 ml
Rnase A (50 mg/ml)	50 μl	150 μl
SP Columns	100 pcs	300 pcs
Collection Tubes	100 pcs	300 pcs

Quality Control

In accordance with BioDiamond's ISO-certified Total Quality Management System, the quality of the BioDiamond Plasmid DNA Miniprep Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

＊Ethanol (96~100%)

＊1.5 ml microcentrifuge tubes

NOTE

★ Add the provided RNase A solution to Buffer S1, mix, and store at 2–8°C.

★ Add ethanol (96–100%) to Buffer W2, **shaking before use** (see bottle label for volume).

★ Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.

★ Buffers S2, S3, and W1 contain irritants. Wear gloves when handling these buffers..

Protocol

Step 1 Bacterial Cells Harvesting

- Transfer 1.5 ml bacterial culture to a microcentrifuge tube
- Centrifuge at 14,000 x g for 1 minute and discard the supernatant.

Step 2 Resuspend

- Resuspend pelleted bacterial cells in 200 μl **Buffer S1** (RNase A added)

Step 3 Lysis

- Add 200 μl **Buffer S2** and mix thoroughly by inverting the tube 10 times (Do not vortex) and then stand at room temperature for 2 minutes or until the lysate is homologous.

Step 4 Neutralization

- Add 300 μl **Buffer S3** and mix immediately and thoroughly by inverting the tube 10 times (Do not vortex). Centrifuge at 14,000 x g for 3 minutes.

Step 5 Binding

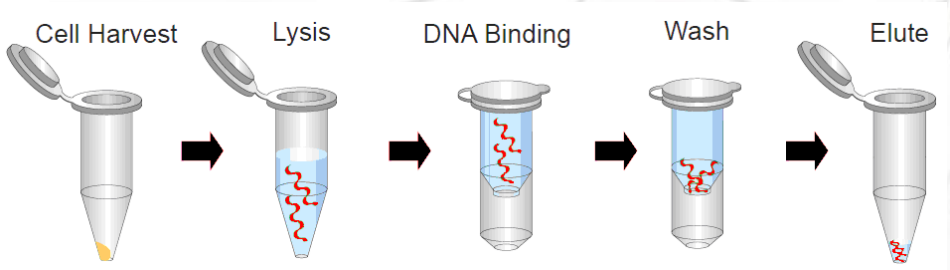
- Place a **SP Column** in a **Collection Tube**. Apply the supernatant (from step 4) to the **SP column** by decanting or pipetting.
- Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the **SP column** back into the same **Collection Tube.**

Step 6 Wash

- Add 400 μl of **Buffer W1** into the **SP Column**. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the **SP column** back into the same **Collection tube**.
- Add 600 μl of **Buffer W2** (Ethanol added) into the **SP Column**. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the **SP column** back into the same **Collection tube.**

Step 7 Elution

- To elute DNA, place the **SP column** in a clean 1.5 ml microcentrifuge tube.
- Add 50-200 μl **Buffer EL** or water (pH is between 7.0 and 8.5) to the center of each **SP column**, let stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.



Troubleshooting

Problem	Cause	Solution
Presence of RNA	RNA contamination	Prior to using Buffer S1, ensure RNase A is added.
Plasmid bands was smeared on agarose gel	Plasmid DNA degradation	Keep plasmid preparations on ice or frozen in order to avoid the plasmid DNA degradation
Presence of genomic DNA	Genomic DNA contamination	Do not overgrow bacterial cultures. Do not incubate more than 5 min after adding the Buffer S1.
Low yields of DNA	Low plasmid copy number	Increase the culture volume. Change the culture medium.
	96~100% ethanol not used	Add ethanol (96~100%) to the Buffer W2 before use.
	Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use the new glass- and plastic-wares, and wear the gloves.
	Column overloaded	Decrease the loading volume or lower the culture density.
	SDS in the Buffer S2 precipitated	The SDS in the Buffer S2 may precipitate with time. If this happens, incubate the Buffer S2 at 30~40°C for 5 min and mix well.
	Incorrect elution conditions	Ensure that the Buffer E is added into the center of the SP Column.
	Plasmid lost in the host *E. coli*	Prepare and use a fresh culture.
	TE buffer used for DNA elution.	Use ethanol to precipitate the DNA, or repurify the DNA fragments and elute with nuclease-free water.
	Presence of residual ethanol in plasmid.	Following the Wash Step, dry the SP Column with additional centrifugation at 14~16,000 x g for 2 minutes.
	Column overloaded	Check the culture volume. If overgrown, add additional reaction buffer. Check the loading volume.
	Inappropriate salt or pH conditions in buffers	Ensure that any buffer prepared in the laboratory was prepared according to the instructions.
	Incomplete removal of the ethanol	Make sure that no residual ethanol remains in the membrane before eluting the plasmid DNA. Re-centrifuge or vacuum again if necessary.

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