Plasmid DNA Midiprep Kit

For research use only

Cat No. DMDPD020& DMDPD050

Sample: up to 100 ml bacterial cells

Yield: up to 250μg of plasmid

Introduction

The BioDiamond Plasmid DNA Midiprep Kit provides a fast, simple, and cost-effective plasmid midiprep method for isolation of plasmid DNA from cultured bacterial cells. The BioDiamond Plasmid DNA Midiprep 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 BioDiamond Plasmid DNA Midiprep 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 40-50 minutes.

Kit Contents

Catalog No.	DMDPD020	DMDPD050
M1 Buffer	85 ml	210 ml
M2 Buffer	85 ml	210 ml
M3 Buffer	125 ml	250 ml, 60 ml
W1 Buffer	125 ml, 40 ml	250 ml, 160 ml
W2 Buffer (Add Ethanol)	25 ml × 2 (100 ml × 2)	50 ml × 2, 25 ml (200 ml × 2, 100 ml)
EL Buffer	50 ml	110 ml
Rnase A (50 mg/ml)	200 μl	500 μl
MP Columns	20 pcs	50 pcs

Quality Control

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

Additional requirements

＊Ethanol (96~100%)

＊50 ml centrifuge tubes

NOTE

★ Add the provided RNase A solution to Buffer M1, mix, and store at 2–8°C.

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

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

★ Buffers M2, M3, and W1 contain irritants. Wear gloves when handling these buffers.

Protocol

Step 1 Bacterial Cells Harvesting

◆Transfer 50~100 ml bacterial culture to a centrifuge tube.

◆Centrifuge at 6,000 x g for 5 minute and discard the supernatant.

Step 2 Resuspend

◆Resuspend pelleted bacterial cells in 4 ml **Buffer M1** (RNase A added)

Step 3 Lysis

◆Add 4 ml **Buffer M2** 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 6 ml **Buffer M3** and mix immediately and thoroughly by inverting the tube 10 times (Do not vortex). Centrifuge at 6,000 x g for 10 minutes.

Step 5 Binding

◆Place a **MP Column** in a 50 ml centrifuge tube. Apply the supernatant (from step 4) to the **MP column** by decanting or pipetting.

◆Centrifuge at 6,000 x g for 3 minutes. Discard the flow-through and place the **MP column** back into the same 50 ml centrifuge tube**.**

Step 6 Wash

◆Add 8 ml of **Buffer W1** into the **MP Column**. Centrifuge at 6,000 x g for 3 minutes. Discard the flow-through and place the **MP column** back into the same 50 ml centrifuge tube.

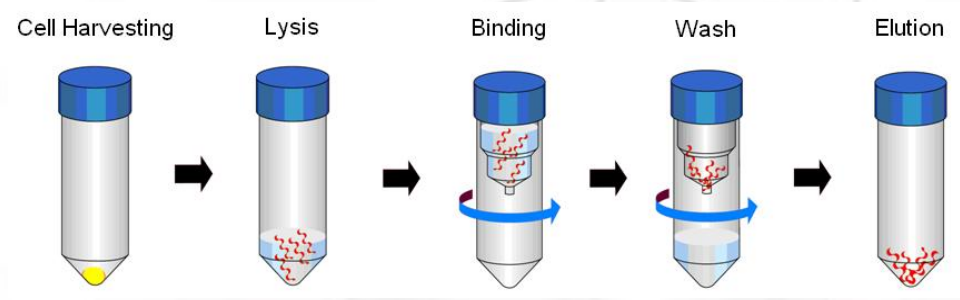
◆Add 12 ml of **Buffer W2** (Ethanol added) into the **MP Column**. Centrifuge at 6,000 x g for 3 minutes. Discard the flow-through and place the **MP column** back into the same 50 ml centrifuge tube.

◆Centrifuge at 6,000 x g again for 3 minutes to remove residual **Buffer W2**.

Step 7 Elution

◆To elute DNA, place the **MP column** in a new 50 ml centrifuge tube.

◆Add 2 ml **Buffer EL** or water (pH is between 7.0 and 8.5) to the center of each **MP column**, let stand for 2 minutes, and centrifuge at 6,000 x g for 3 minutes.



Troubleshooting

Problem	Cause	Solution
Presence of RNA	RNA contamination	Prior to using Buffer M1, 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 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 M1.
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 new glass- and plastic-wares, and wear gloves.
	Column overloaded	Decrease the loading volume or lower the culture density.
	SDS in the Buffer S2 precipitated	The SDS in Buffer M2 may precipitate with storage. If this happens, incubate the Buffer M2 at 30~40°C for 5 min and mix well.
	Incorrect elution conditions	Ensure that Buffer E is added into the center of the MP Column.
	Plasmid lost in the host *E. coli*	Prepare and use 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 MP Column with an additional centrifugation step at 6,000 x g for 5 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 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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