Plasmid *midi*PREP Kit

Cat No. NA205-0020 Size: 20 Reactions
Cat No. NA205-0002 Size: 2 Reactions
Sample: Up to 100 ml bacterial cells
Yield: Up to 250 µg of plasmid
Endotoxin value: <0.003 EU/µg
Operation time: Within 40 minutes
Elution volume: 2 ml

Description

The Plasmid *midi*PREP Kit provides a fast, simple, and cost-effective method for the plasmid DNA isolation from the cultured bacterial cells. The Plasmid *midi*PREP Kit is based on the alkaline lysis of bacterial cells, followed by binding DNA onto the glass fiber matrix of the spin column in the presence of high salt. Phenol extraction and ethanol precipitation are not required, and the high-quality plasmid DNA is eluted in a small volume of the Tris buffer (included in each kit) or water (pH is between 7.0 and 8.5). The plasmid DNA purified with the Plasmid *midi*PREP Kit is suitable for a variety of routine applications, including the restriction enzyme digestion, sequencing, library screening, in vitro translation, transfection of robust cells, ligation, and transformation. The entire procedure can be completed within 40 minutes.

Features

- Ready-to-use DNA for high performance in any downstream application.
- Consistent DNA yields from a small amount of the starting material.
- Time flexibility.

Applications

- Quantity of DNA needed.
- Molecular weight and size of DNA.
- Purity of DNA required.

Kit Contents

Contents	NA205-0020	NA205-0002
Buffer M1	85 ml X 1 bottle	10 ml X 1 bottle
Buffer M2	85 ml X 1 bottle	10 ml X 1 bottle
Buffer M3	125 ml X 1 bottle	15 ml X 1 bottle
Buffer W1	125 ml X 1 bottle, 40 ml X 1 bottle	20 ml X1 bottle
Buffer W2 (Add ethanol)	25 ml x 2 bottles (100 ml X 2 bottles)	6 ml X 1 bottle (24 ml X 1 bottle)
Buffer BE	50 ml X 1 bottle	5 ml X 1 bottle
RNase A (50mg/mL)	200 µl	Has been added into Buffer M1
MD Column	20 pieces X 1 bag	2 pieces X 1 bag

Quality Control

The quality of the Plasmid *midi*PREP Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Ethanol (96-100%)
- Microcentrifuge tubes

Buffer Preparation

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

Plasmid *midi*PREP Kit Protocol

Step 1 Bacterial Cells Harvesting

1. Transfer 50 ml of the bacterial culture to a 50 ml centrifuge tube.
2. Centrifuge at 6,000 x g for 5 minute and discard the supernatant.

Step 2 Resuspension

1. Resuspend pelleted bacterial cells in 4 ml of the Buffer M1 (RNase A added)

Step 3 Lysis

1. Add 4 ml of the Buffer M2 and mix thoroughly by inverting the tube 10 times (Do not vortex) and then stand at the room temperature for 2 minutes or until the lysate is homologous.

Step 4 Neutralization

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

Step 5 Binding

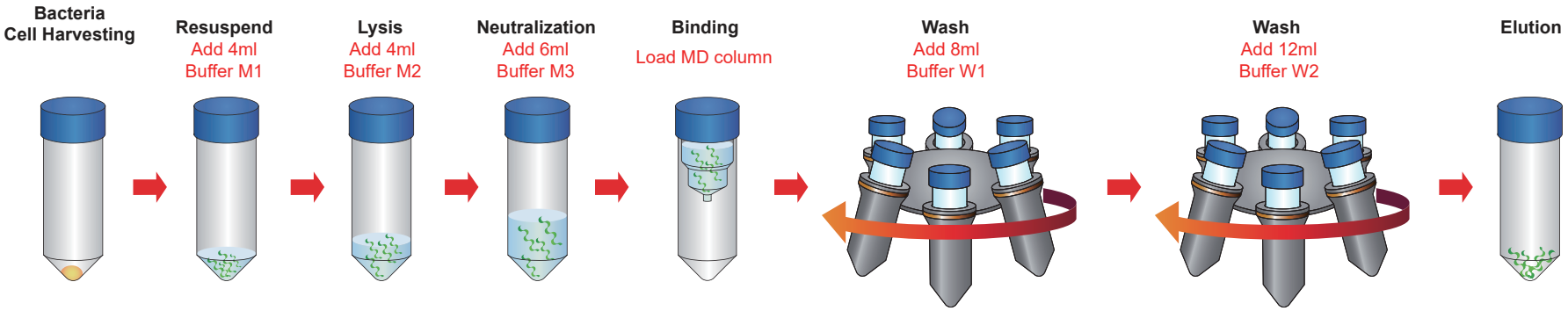
1. Place a MD Column in a 50 ml centrifuge tube.
2. Apply the supernatant (from step 4) to the MD column by decanting or pipetting.
3. Centrifuge at 6,000 x g for 3 minutes.
4. Discard the flow-through and place the MD column back into the same 50 ml centrifuge tube.

Step 6 Wash

1. Add 8 ml of the Buffer W1 into the MD Column.
2. Centrifuge at 6,000 x g for 3 minutes.
3. Discard the flow-through and place the MD column back into the same 50 ml centrifuge tube.
4. Add 12 ml of the Buffer W2 (Ethanol added) into the MD Column.
5. Centrifuge at 6,000 x g for 3 minutes.
6. Discard the flow-through and place the MD column back into the same 50 ml centrifuge tube.
7. Centrifuge at 6,000 x g again for 3 minutes to remove residual Buffer W2.

Step 7 Elution

1. To elute DNA, place the MD column in a new 50 ml centrifuge tube.
2. Add 2 ml of the Buffer BE or water (pH is between 7.0 and 8.5) to the center of each MD column, let it stand for 2 minutes, and centrifuge at 6,000 x g for 3 minutes.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did genomic DNA isolation with the kit.

Problem	Cause	Solution
Presence of RNA	RNA contamination	Prior to using the 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 the plasmid DNA degradation.
Presence of genomic DNA	Genomic DNA contamination	1. Do not overgrow bacterial cultures. 2. Do not incubate more than 5 min after adding the Buffer M1.
Low yields of DNA	96-100% ethanol not used	Add ethanol (96-100%) to the Buffer W2 before use.
	Nuclease contamination	1. Check buffers for nuclease contamination and replace if necessary. 2. 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 M2 precipitated	The SDS in the Buffer M2 may precipitate upon storage. If this happens, incubate the Buffer M2 at 30-40°C for 5 min and mix well.
	Incorrect elution conditions	Ensure that the Buffer BE is added into the center of the MD Column.
	Plasmid lost in the host	Prepare the fresh culture.
	TE buffer used for DNA elution.	Use the ethanol to precipitate the DNA, or repurify the DNA fragments and elute with the nuclease-free water.
Inhibition of downstream enzymatic reactions	Presence of residual ethanol in plasmid	Following the Wash Step, dry the MD Column with the additional centrifugation at 6,000 x g for 3 minutes
DNA passed through in the flow-through or wash fraction	Inappropriate salt or pH conditions in buffers	Ensure that any buffer prepared in the laboratory was prepared according to the instructions.
Plasmid DNA floats out of wells while running in agarose gel	Incomplete removal of the ethanol	1. Make sure that no residual ethanol remains in the membrane before eluting the plasmid DNA. 2. Re-centrifuge or vacuum again if necessary

Related Ordering information

Cat. No.	Description	Size
MB101-0500	*Taq* DNA polymerase	500 U
MB201-0100	Hot Start SUPERMIX	100 Reactions
AGT001-0500	AGAROSE Tablet, 0.5g	100 Tablets
LD001-1000	Novel Juice Supplied in 6X Loading Buffer	1 ml

Caution

- Check buffers before use for salt precipitation.
- Re-dissolve any precipitate by warming up to 37°C.
- Buffers M2, M3 and W1 contain irritants. Wear gloves when handling these buffers.
- When using 20 reaction assays, add 100 ml of the ethanol (96-100%) to each bottle of the Buffer W2, and shake before use.
- When using 2 reaction assays, add 24 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.