Plasmid *maxi*PREP Kit

Cat No. PDP03-0010
Size: 10 Reactions
Sample: Up to 200 ml bacterial cells
Yield: Up to 850 µg of plasmid
Endotoxin value: <0.003 EU/ug

Description

The Plasmid *maxi*PREP Kit provides a fast, simple, and cost-effective plasmid maxiprep method for isolating the plasmid DNA from the cultured bacterial cells. The Plasmid *maxi*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 *maxi*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.

Kit Contents

Contents	NA305-0010
Buffer M1	85 ml
Buffer M2	85 ml
Buffer M3	125 ml
Buffer W1	105 ml
Buffer W2 *Add 100 ml of ethanol prior to initial use.	25 ml
Buffer E	30 ml
RNase A (50mg/ml)	200 ul
MX Column	10 pcs

Required Materials

- Ethanol (96～100%)
- 50 ml centrifuge tubes

Buffer Preparation

- Add the provided RNase A solution to the Buffer M1, mix, and store at 2~8°C.
- Add 100 ml of ethanol (96~100%) to the Buffer W2 before use.

Applications

- Transfection
- Microinjection
- Sequencing
- PCR
- Restriction enzyme digestion

Plasmid *maxi*PREP Kit Protocol

Step 1 Bacterial Cells Harvesting

1. Transfer 200 ml of the bacterial culture to a centrifuge tube.
2. Centrifuge at 6,000 x g for 5 minute and discard the supernatant.

Step 2 Resuspend

1. Resuspend the pelleted bacterial cells in 8 ml of the **Buffer M1** (RNase A added)

Step 3 Lysis

1. Add 8 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 12 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

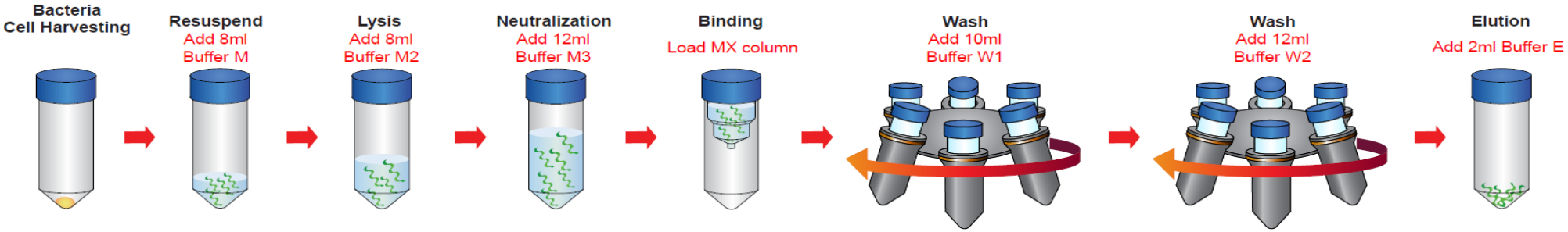
1. Place a **MX Column** in a 50 ml centrifuge tube.
2. Apply 15 ml of the supernatant (from step 4) to the **MX Column** by decanting or pipetting.
3. Centrifuge at 6,000 x g for 3 minutes.
4. Discard the flow-through and place the **MX Column** back into the same 50 ml centrifuge tube.
5. Transfer the remaining supernatant to the same **MX Column**.
6. Centrifuge at 6,000 x g for 3 minutes.
7. Discard the flow-through and place the **MX Column** back into the same 50 ml centrifuge tube.

Step 6 Wash

1. Add 10 ml of the **Buffer W1** into the **MX Column**.
2. Centrifuge at 6,000 x g for 3 minutes.
3. Discard the flow-through and place the **MX Column** back into the same 50 ml centrifuge tube.
4. Add 12 ml of the **Buffer W2** (Ethanol added) into the MX Column.
5. Centrifuge at 6,000 x g for 3 minutes.
6. Discard the flow-through and place the **MX Column** back into the same 50 ml centrifuge tube.
7. Centrifuge at 6,000 x g again for 3 minutes to remove the residual **Buffer W2**.

Step 7 Elution

1. To elute DNA, place the **MX Column** in a new 50 ml centrifuge tube.
2. Add 2 ml of the **Buffer E** or water (pH is between 7.0 and 8.5) to the center of each **MX 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 purifying plasmid DNA with the kit.

Problem	Cause	Solution
Presence of RNA	RNA contamination	Prior to using the Buffer M1, ensure that the RNase A is added.
Smeared plasmid bands on the agarose gel	plasmid DNA degradation	Keep plasmid preparations on ice or frozen in order to avoid the plasmid DNA degradation.
Poor plasmid quality	Genomic DNA contamination	Do not overgrow bacterial cultures. Do not incubate more than 5 min after adding the Buffer M1.
Low yields of DNA	Insufficient performance of the elution buffer during the elution step	Remove residual wash buffers during the Wash Step completely. Remaining buffers decrease the efficiency of the following wash steps and elution step.
	Low copy number of plasmid.	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 ware, and wear gloves.
	Column overloaded	Decrease the loading volume or lower the culture density.
	SDS in the Buffer M2 precipitated	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 E is added into the center of the MX Column.
	Plasmid lost in the host *E. coli*	Prepare the fresh culture.
Inhibition of downstream enzymatic reactions	Presence of residual ethanol in plasmid	Remove EtOH in the hood briefly Following the Wash step, dry the MX Column with additional centrifugation at 14~6,000 x g for 3 minutes.
DNA passed through in the flow-through or wash fraction	Column overloaded	Check the culture volume. If overgrown, add the reaction buffer. Check the loading volume.
	Inappropriate salt or pH conditions in buffers	Ensure that any buffers prepared in the laboratory were prepared according to the instructions.
Plasmid DNA floats out of wells while running on the agarose gel	Traces of ethanol not completely removed from the column	Make sure that no residual ethanol remains in the membrane before eluting the plasmid DNA. Re-centrifuge again if necessary.

Quality Control

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

Features

- Safe: Eliminates the use of phenol, chloroform, ethidium bromide, and cesium chloride, thus minimizing the exposure to and disposal of hazardous materials.
- Time saving: Complete the process in less than 40 minutes.

Caution

- Check buffers before use for salt precipitation.
  Re-dissolve any precipitate by warming up to 37°C.
- Add the provided RNase A solution to the Buffer M1, mix, and store at 2~8°C.
- Add 100 ml of ethanol (96~100%) to the Buffer W2 before use.
- Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming to 37°C.
- Buffers M2, M3, and W1 contain irritants. Wear gloves when handling these buffers.
- 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.