# Plasmid *midi* PREP Kit

Cat No. PDP02-0020(S)
Size: 20 Reactions
Sample: Up to 100 ml bacterial cells
Yield: Up to 250 μg of plasmid
Endotoxin value: <0.003 EU/μg

## Description

The Plasmid *midi*PREP Kit provides a fast, simple, and cost-effective plasmid midiprep method for isolating the plasmid DNA 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.

## Kit Contents

| Contents | PDP02-0020 | PDP02-0020S |
|---|---|---|
| Buffer M1 | 85 ml | 10 ml |
| Buffer M2 | 85 ml | 10 ml |
| Buffer M3 | 125 ml | 15 ml |
| Buffer W1 | 125 ml, 40 ml | 20 ml |
| Buffer W2<br>*Add 100 ml of the ethanol (96~100%) to each bottle of the Buffer W2 before use. | 25 ml x 2 | 6 ml |
| Buffer E | 50 ml | 5 ml |
| RNase A (50mg/ml) | 200 μl | Added |
| MD Column | 20 pcs | 2 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

The purified plasmid DNA can be immediately used in any downstream molecular biology application.

- Transfecttion
- Sequencing
- Restriction enzyme digestion
- Microinjection
- PCR

# 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 Resuspend

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.

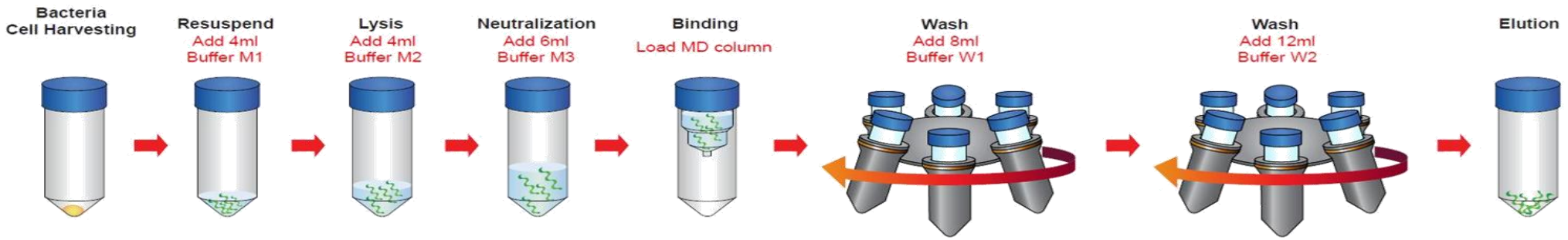
## Step 6 Wash

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 E** 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.

# Protocol




## 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.<br>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.<br>Remaining buffers decrease the efficiency of the following wash steps and elution step. |
| | Low copy-number of plasmid. | Increase the culture volume.<br>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.<br>Use new glass- and plastic ware, and wear gloves. |
| | Column overloaded | Decrease the loading volume or lower the culture density. |
| | SDS in Buffer M2 precipitated | SDS in Buffer M2 may precipitate upon storage. If this happens, incubate 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 MD Column. |
| | Plasmid lost in the host *E. coli* | Prepare the fresh culture. |
| Inhibition of downstream enzymatic reactions | Presence of residual ethanol in plasmid | Remove ethanol in the hood briefly.<br>Following the Wash step, dry the MD Column with additional centrifugation at 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.<br>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 column | Make sure that no residual ethanol remains in the membrane before eluting the plasmid DNA.<br>Re-centrifuge again if necessary. |

## Quality Control

The quality of the Plasmid *midi*PREP 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 Buffer M1, mix, and store at 2~8°C.
- Add 100 ml of the ethanol (96~100%) to each bottle of the Buffer W2 before use.
- 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.
- 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.

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