Plasmid *mini*PREP Kit

Cat No. PDP01-0100(S)
Size: 100 Reactions

Description

The **Plasmid *mini*PREP Kit** provides a fast, simple, and cost-effective plasmid miniprep method for isolating the plasmid DNA from the cultured bacterial cells. The Plasmid *mini*PREP Kit is based on the alkaline lysis of bacterial cells, followed by the binding of the DNA onto the glass fiber matrix of the spin column in the presence of high salt. The phenol extraction and ethanol precipitation are not required, and the high-quality plasmid DNA is eluted in a small volume of Tris buffer (included in each kit) or water (pH between 7.0 and 8.5). The plasmid DNA purified with the Plasmid *mini*PREP 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 15~20 minutes.

Kit Contents

Contents	PDP01-0100	PDP01-0100S
Buffer S1	25 ml	1 ml
Buffer S2	25 ml	1 ml
Buffer S3	35 ml	1.5 ml
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	300 µl (1.2 ml)
Buffer E	10 ml	1 ml
RNase A (50mg/ml)	50 µl	Added
PM Columns	100 pcs	4 pcs
Collection Tubes	100 pcs	4 pcs

Quality Control

The quality of the Plasmid *mini*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 S1, mix, and store at 2~8°C.
- Add 60ml of ethanol (96~100%) to the Buffer W2 before use.

Protocol

Step 1 Bacterial Cells Harvesting

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

Step 2 Resuspend

1. Resuspend pelleted bacterial cells in 200 µl of the Buffer S1 (RNase A added).

Step 3 Lysis

1. Add 200 µl of the Buffer S2 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 300 µl of the Buffer S3 and mix immediately and thoroughly by inverting the tube 10 times (Do not vortex).
2. Centrifuge at 14,000 x g for 3 minutes.

Step 5 Binding

1. Place a PM column in a Collection Tube. Apply the supernatant (from step 4) to the PM column by decanting or pipetting.
2. Centrifuge at 14,000 x g for 30 seconds, then discard the flow-through, and place the PM column back into the same collection tube.

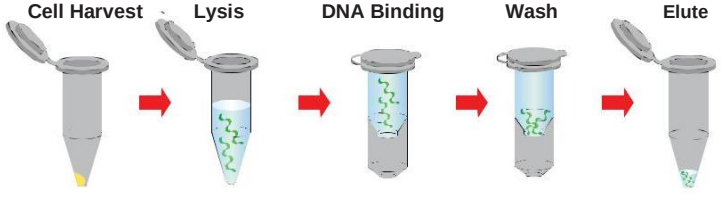
Step 6 Wash

1. Add 400 µl of the Buffer W1 into the PM column.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the PM column back into the same collection tube.
4. Add 600 µl of the Buffer W2 (Ethanol added) into the PM column.
5. Centrifuge at 14,000 x g for 30 seconds.
6. Discard the flow-through and place the PM column back into the same collection tube.
7. Centrifuge at 14,000 x g again for 2 minutes to remove the residual Buffer W2.

Step 7 Elution

1. To elute DNA, place the PM column in a clean 1.5 ml microcentrifuge tube.
2. Add 50~100 µl of the Buffer E or H2O (pH between 7.0 and 8.5) to the center of each PM column, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.

＊Check the buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.



BIO-HELIX CO., LTD.
5F., No. 145, Sec. 3, Beixin Rd., Xindian Dist., New Taipei City, Taiwan (R.O.C)
Tel: +866-2-24624956 Fax: +866-2-24628849
PureDireX

Troubleshooting

Refer to the table below to troubleshooting 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 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 upon storage. 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 PM Column.
	Plasmid lost in the host *E. coli*	Prepare the fresh culture.
Inhibition of downstream enzymatic reactions	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.
	Presence of residual ethanol in plasmid	Following the Wash Step, dry the PM Column with the additional centrifugation at 14~16,000 x g for 2 minutes.
DNA passed through in the flow-through or wash fraction	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.
Plasmid DNA floats out of wells while running in agarose gel	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.

Caution

- Check buffers before use for salt precipitation. Redissolve any precipitate by warming up to 37°C.
- Buffers S2, S3 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.