Plasmid *mini*PREP Kit

Cat. No.: SN005-0300 Size: 300 Reactions
Cat. No.: SN005-0100 Size: 100 Reactions
Cat. No.: SN005-0004 Size: 4 Reactions
Sample: Up to 1.5 ml of bacterial cells
Format: column form
Operation time: 15-20 minutes
Elution volume: 50-200 µl

Description

The Plasmid *mini*PREP Kit provides a fast, simple, and cost-effective method for the plasmid DNA isolation 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 the kit) or water (pH between 7.0 and 8.5, not provided in the kit). 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.

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

Kit Contents

Contents	SN005-0300	SN005-0100	SN005-0004
Buffer S1	65 ml X 1 bottle	25 ml X 1 bottle	1 ml X 1 vial
Buffer S2	65 ml X 1 bottle	25 ml X 1 bottle	1 ml X 1 vial
Buffer S3	95 ml X 1 bottle	35 ml X 1 bottle	1.5 ml X 1 vial
Buffer W1	125 ml X 1 bottle	45 ml X 1 bottle	2 ml X 1 vial
Buffer W2 (Add ethanol)	25 ml X 2 bottles (100 ml X 2 bottles)	15 ml X 1 bottle (60 ml X 1 bottle)	0.3 ml X 2 vials (1.2 ml X 2 vials)
Buffer BE	30 ml X 1 bottle	10 ml X 1 bottle	1 ml X 1 vial
RNase A (50mg/mL)	150 µl X 1 vial	50 µl X 1 vial	Has been added into Buffer S1
PM Column	50 pieces X 6 bags	50 pieces X 2 bags	4 pieces X 1 bag
Collection Tube	50 pieces X 6 bags	50 pieces X 2 bags	

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.

Plasmid *mini*PREP Kit Protocol

Step 1 Bacterial Cells Harvesting

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

Step 2 Resuspension

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

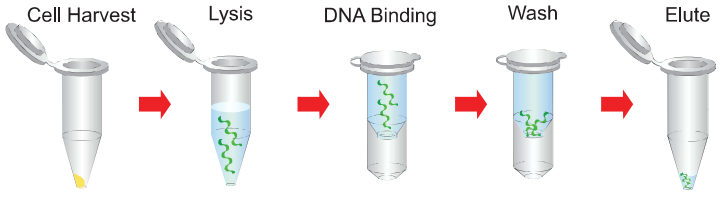
1. Place a PM column in a Collection Tube. Apply the supernatant (from step 4) to the PM column by decanting or pipetting.
2. Centrifuge at 14,000 x g for 30 seconds, then discard the flow-through, and place the PM column back into the same Collection Tube.

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. Add 50-200 µl of the Buffer BE or H_2O (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.



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Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did plasmid DNA isolation 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 condition	Ensure that the Buffer BE is added into the center of the PM Column.
	Plasmid lost in the host	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

Related Order Information

Cat. No.	Description	Size
SM101-0500	*Taq* DNA polymerase	500 U
SM201-0100	Hot Start SUPERMIX	100 Reactions
SL001-1000	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 S2, S3 and W1 contain irritants. Wear gloves when handling these buffers.
> When using 300 reaction assays, add 100 ml of the ethanol (96-100%) to each bottle of the Buffer W2, and shake before use.
> When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
> When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to each vial of the Buffer W2, and shake before use.
> Buffers W1 contain irritants. Wear gloves when handling these buffers.
> During operation, always wear a lab coat, disposable gloves, and protective equipment.
V All products are for research use only.