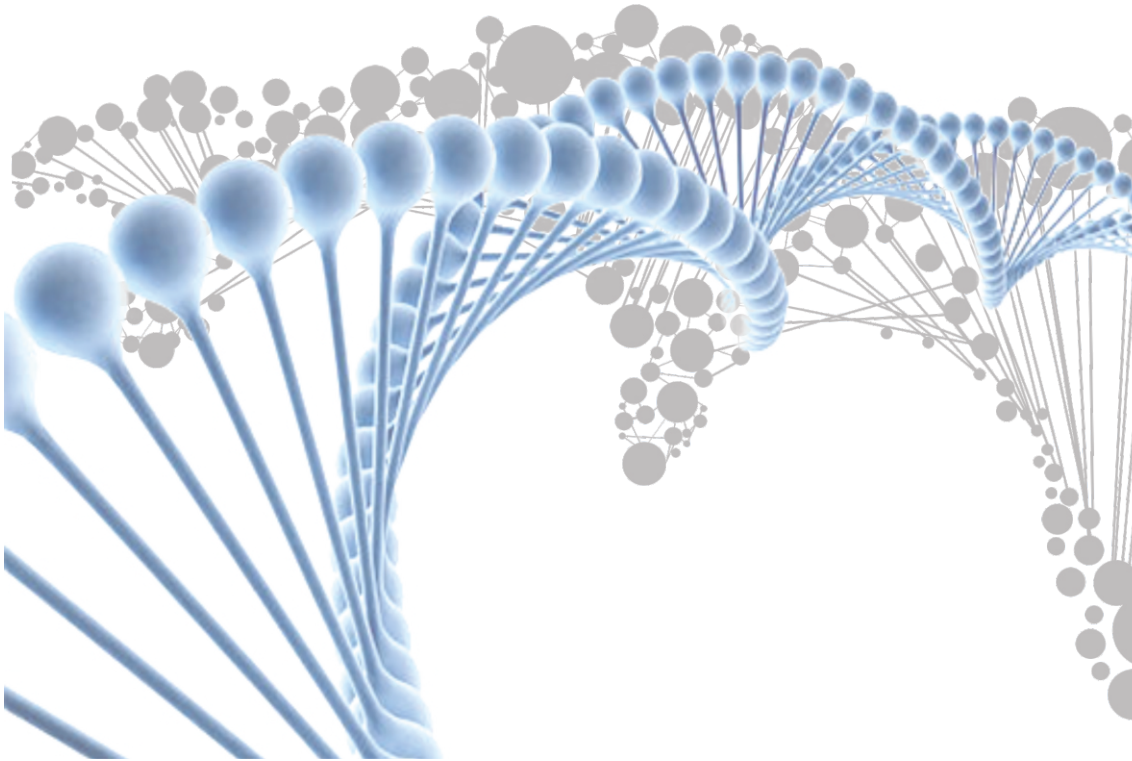
MPD-01100/300

Plasmid Miniprep Kit



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Plasmid Maxiprep Kit

Storage: Stored at room temperature (15-25°C).
Format: spin column **For research use only**
Sample: about 3-5ml cultured cell **Yield: up to 30μg of plasmid**

Introduction:

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

About the kits:

Cat. No.	MPD-01100	MPD-01300
SN Columns/ Tubes	100 pcs	300 pcs
Solution 1	25 ml	65 ml
Solution 2	25 ml	65 ml
Solution 3	35 ml	95 ml
W1 Buffer	45 ml	125 ml
W2 Buffer	15 ml	25 ml x2
RNase A (50mg/ ml)	50 μl	150 μl
Elution Buffer	10 ml	30 ml

NOTE:

1. Add the provided RNase A solution to Solution 1 and mix well then store at 2–8°C.
2. Add ethanol (96–100%) to W2 Buffer before use (see bottle label for volume).
3. If necessary, re-dissolve any precipitate by warming to 37°C.
4. Solution 2, 3 and Buffers W1 contain irritants. Wear gloves when handling these buffers.

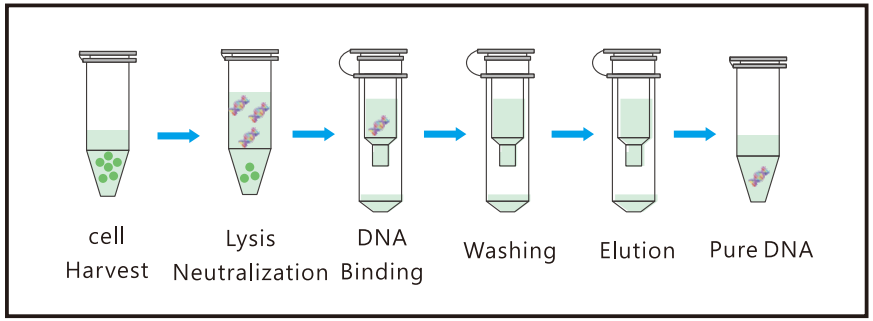
Troubleshooting

Problem	Comments and suggestions
DNA passed through in the flow-through or wash fraction	
>Column overloaded	1. Check the culture volume. If overgrown, add additional reaction buffer. 2. Check the loading volume.
>Inappropriate salt or pH conditions in buffers	Ensure that any buffer prepared in the laboratory was prepared according to 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 product and Ordering information:

CAT NO:	Product	Quantity
MGK-T0100	EBL Genomic DNA Isolation Kit (Tissue)	100 rxn.
MGK-T0300	EBL Genomic DNA Isolation Kit (Tissue)	300 rxn.
MGK-C0100	EBL Genomic DNA Isolation Kit (Cell/ Blood)	100 rxn.
MGK-C0300	EBL Genomic DNA Isolation Kit (Cell/ Blood)	300 rxn.
MGK-P0100	EBL Genomic DNA Isolation Kit (Plant)	100 rxn.
MGK-P0300	EBL Genomic DNA Isolation Kit (Plant)	300 rxn.
MPD-01300	EBL Plasmid Miniprep DNA Kit	300 rxn.
MPD-02025	EBL 200PLUS Plasmid Midiprep DNA Kit	25 rxn
MPD-03020	EBL 800PLUS Plasmid Maxiprep DNA Kit	20 rxn
FBRE100	Blood/ cell Total RNA Isolation kit	100 rxn.
FBRT100	Tissue Total RNA Isolation kit	100 rxn.
FBRP	Plant Total RNA Isolation kit	100 rxn.

Procedure:



Protocol:

Cultured Cells Harvesting

1. Transfer 3-5 ml bacterial culture to a centrifuge tube.
2. Centrifuge at 14,000 x g for 1 minute and discard the supernatant.

Resuspend

3. Resuspend bacterial cells pellet in 200 µl of Solution 1.

 # Add the provided RNase A solution to Solution 1 and mix well then store at 2–8°C.

Lysis

4. Add 200 µl of Solution 2 and mix thoroughly by inverting the tube 10 times (Do not vortex) and then stand at room temperature for 2 minutes or until the lysate is homologous.

Neutralization

5. Add 300 µl of Solution 3 and mix immediately and thoroughly by inverting the tube 10 times (Do not vortex). Centrifuge at 14,000xg for 5 minutes.

Binding

6. Place a SN Column in a Collection Tube. Apply the supernatant (from step 5) to the SN column by decanting or pipetting..
7. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the SN column back into the same Collection Tube.

Wash

8. Add 400 µl of W1 Buffer into the SN Column. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the SN column back into the same Collection tube.
9. Add 600 µl of W2 Buffer (Ethanol added) into the SN Column. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the SN column back into the same Collection tube.
10. Centrifuge at 14,000 x g again for 2 minutes to remove residual W2Buffer.

Elution

11. To elute DNA, place the SN column in a clean 1.5 ml microcentrifuge tube.
12. Add 50-200 µl Elution Buffer or water (pH is between 7.0 and 8.5) to the center of each SN column, let stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.

Troubleshooting

Problem	Comments and suggestions
Presence of RNA	
>RNA contamination	Ensured RNase A is added before using Solution 1.
Plasmid bands was smeared on agarose gel	
>plasmid DNA degradation	Keep plasmid preparations on ice or frozen in order to avoid plasmid DNA degradation.
Presence of genomic DNA	1. Do not overgrow bacterial cultures.
>Genomic DNA contamination	2. Do not incubate more than 5min after added Solution 1.
Low yields of DNA	
>Low plasmid copy number	1. Increase the culture volume. 2. Change the culture medium.
>96~100% ethanol not used	Add ethanol (96～100%) to the W2 Buffer before use.
>Nuclease contamination	1. Check buffers for nuclease contamination and replace if necessary. 2. Use new 1.5 ml centrifuge tube and wear gloves.
>Column overloaded	Decrease the loading volume or lower the culture density.
>SDS in the Solution 2 precipitated	The SDS in Solution 2 may precipitate with storage. If this happens, incubate the Solution 2 at 30~40°C for 5 min and mix well.
>Incorrect elution conditions	Ensure that Elution Buffer is added into the center of the SN Column.
>Plasmid lost in the host E. coli	Prepare and use 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 SN Column with an additional centrifugation step at 14,000 x g for 2 minutes.