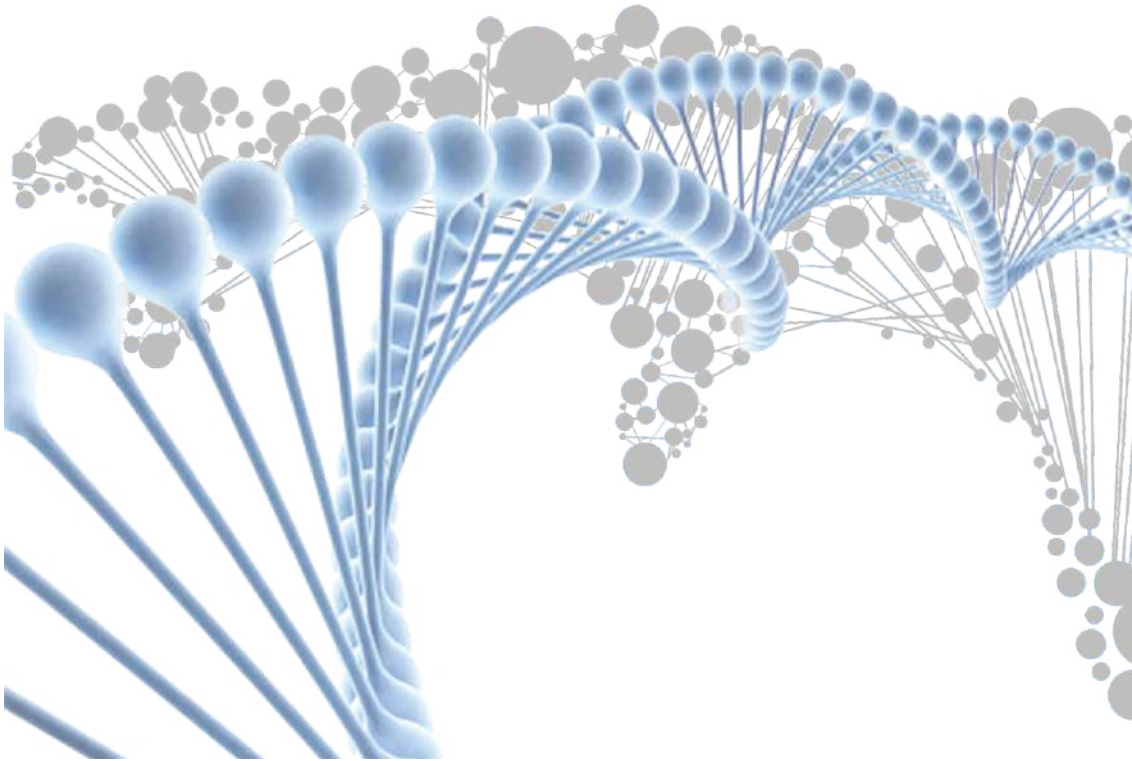
MPD-02020

Plasmid Midiprep Kit



www.eblbio.com

Plasmid Midiprep Kit

Storage: Stored at room temperature (15-25°C).
Format: spin column **For research use only**
Sample: about 100ml cultured cell

Introduction:

The EBL Plasmid Midiprep Kit provides a fast, simple, and cost-effective plasmid midiprep method for isolation of plasmid DNA from cultured bacterial cells. The Midiprep 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 Elution/ Tris buffer (included in each kit) or water (pH is between 7.0 and 8.5). Plasmid DNA purified with EBL Plasmid Midiprep 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 40-50 minutes.

About the kits:

Cat. No.	MPD-02020
SM Columns	20 pcs
Solution 1	85 ml
Solution 2	85 ml
Solution 3	125 ml
W1 Buffer	165 ml
W2 Buffer	25 ml x2
RNase A (50mg/ ml)	200 µl
Elution Buffer	50 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.

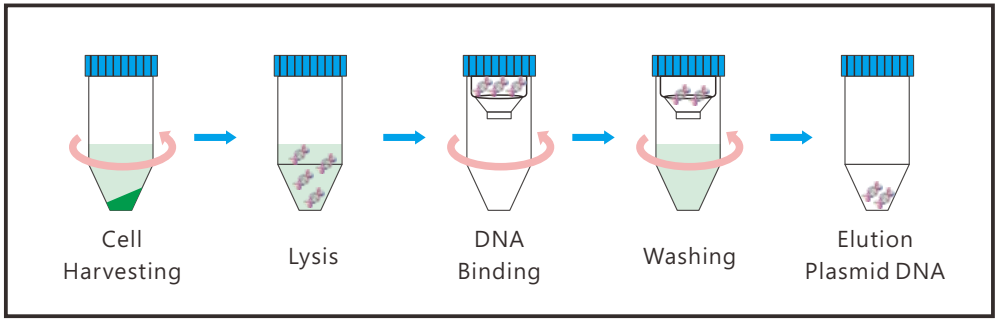
Troubleshooting

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 50-100 ml bacterial culture to a centrifuge tube.
2. Centrifuge at 6,000 x g for 5 minute and discard the supernatant.

Resuspend

3. Resuspend bacterial cells pellet in 4 ml of Solution 1.

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

Lysis

4. Add 4 ml 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 6 ml of Solution 3 and mix immediately and thoroughly by inverting the tube 10 times (Do not vortex). Centrifuge at 6,000xg for 15 minutes.

Binding

6. Place a SM Column in a 50 ml centrifuge tube. Apply 14 ml of the supernatant (Neutralization step) to the SM column by decanting or pipetting.
7. Centrifuge at 6,000 x g for 3 minutes. Discard the flow-through and place the SM column back into the same 50 ml centrifuge tube.

Wash

9. Add 8 ml of W1 Buffer into the SM Column. Centrifuge at 6,000 x g for 3 minutes. Discard the flow-through and place the SM column back into the same 50 ml centrifuge tube.
10. Add 12 ml of W2 Buffer (Ethanol added) into the SM Column. Centrifuge at 6,000 x g for 3 min. Discard the flowthrough and place the SM column back into the same 50 ml centrifuge tube.
11. Centrifuge at 6,000 x g again for 3 minutes to remove residual W2 Buffer.

Elution

12. To elute Plasmid DNA, place the SX column in a new 50 ml centrifuge tube.
13. Add 2 ml Elution Buffer or water to the center of each SM column, let stand for 2 minutes, and centrifuge at 6,000 x g for 3 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 50 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 SM 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 SM Column with an additional centrifugation step at 6,000 x g for 5 minutes.