For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

ISO 9001/14001 Certified Company

DNA-maxi™ SV Plasmid DNA Purification Kit

Cat. No. 17253 12 Columns

DESCRIPTION

DNA-maxi™ SV Plasmid DNA Purification Kit provides easy and rapid method for the medium scale preparation of plasmid DNA from bacterial cells. This kit can be used to isolate and purify any plasmid, also can isolated maximum 40 Kb size plasmid DNA. The plasmid DNA is free from protein, genomic DNA, and RNA contaminants. This pure plasmid DNA is ready for PCR, cloning, automated or manual sequencing, transfection, synthesis of labeled hybridization probes, electroporation, and enzymatic restriction analysis.

KIT CONTENTS and STORAGE

Store at RT except for M1 Buffer. M1 Buffer should be stored at 4℃ after adding RNase A Solution.

- M1 Buffer (Resuspension Buffer) 80 ml
  : Before use, add 4 ml of RNase A solution to M1 Buffer. Then, store at 4℃.
- M2 Buffer (Lysis Buffer) 80 ml
  : Check M2 Buffer for SDS precipitation due to low storage temperature, in which case it is necessary to dissolve the SDS by warming to 37℃.
- M3 Buffer (Neutralization Buffer) 80 ml
- Washing Buffer A 250 ml
  : *endA*$^+$ strains such as HB101, the JM series strains, PR series strains and some other wide-type strains have high endonucleases activity. Endonucleases that can degrade plasmid DNA are essentially removed by Washing Buffer A of DNA-midi™ SV Kit.
- Washing Buffer B 60 ml (30 ml x 2ea)
  : Before use, each 30 ml of Washing Buffer B's bottle , add 120 ml of absolute EtOH.
- Elution Buffer 30 ml
  : DNase / RNase free Ultra-Pure solution.
- RNase A Solution (10mg/ml) 6 ml
  : After receiving, store at 4℃.
- Pre Column (Clear Color) 12 Columns
  : Inserted into the 50 ml disposable tube (collection tube).
- Binding Column (Yellow Color) 12 Columns
  : Inserted into the 50 ml disposable tube (collection tube).

ADDITIONAL REQUIRED EQUIPMENT

- Absolute EtOH
- Vacuum manifold & vacuum pump
- Conventional centrifuge (with swinging-bucket rotor, ability of 3,500~4,000rpm)
- 50ml disposable tube

CHARACTERISTICS

- Easy to use - organic extraction or ethanol precipitation is no required.
- No phenol or chloroform is used.
- Spend only 30 min (vacuum protocol), 80 ~ 90 min (spin protocol) to extract plasmid DNA.
- Cell lysates remove easily with Pre Column.
  : After mixing with M3 Buffer, the cellular debris and precipitates should be removed completely not to clog Binding Column in subsequent binding. Pre Column facilitates the clearance of the lysate by filtration instead of laborious incubation on ice and centrifugation which has been used widely in traditional methods.
- Plasmid DNA binds selectively to silica membrane.
- This column system apply spin and vacuum protocol.

PROTOCOL A (Spin Protocol)

1. Pick a single colony from a freshly streaked bacterial plate and use it to inoculate LB (+antibiotics). And then grow at 37 ℃ for 12 ~ 16 hrs with vigorous shaking (OD_{600} = 1.0 ~ 1.5).
   Note : Use the appropriate volume of bacterial cultures; 100 ~ 150 ml for high copy number plasmid, up to 300 ~ 400 ml for low copy number plasmid.
2. Harvest the bacteria culture by centrifugation for 10 min at 13,000 rpm at 4℃.
   Note : Remove all traces of supernatant by inverting the open tube until all media has drained.
3. Resuspend pelleted bacterial cell thoroughly in 6 ml of M1 Buffer by vortexing until no clumps remain.
   Note : Ensure that RNase A solution has been added to M1 Buffer. It is essential to completely resuspend the cell pellet. It may affect the lysis efficiency.
4. Add 6 ml of M2 Buffer to resuspended cells and mix by inverting the tube 10 times. DO NOT VORTEX and incubate for 3min at RT.
   Note : The optimal lysis time allows maximum release of plasmid DNA without release of chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. Long exposure to alkaline condition may cause the plasmid to become irreversibly denatured. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps. Do not vortex, it may cause shearing of genomic DNA.
5. Add 6 ml of M3 Buffer and gently mix by inverting the tube 10 times.
   Note : After addition of M3 Buffer, the solution should become cloudy and a fluffy white form. Incubation on ice may help precipitate the denatured cell components more efficiently. The precipitated material contains genomic DNA, protein, cell debris, and SDS.
6. Pour lysate into the Pre Column (clear color, inserted into the 50 ml disposable tube). And centrifuge for 10 min at 3,500 rpm (4℃).
   Note : A small amount of insoluble material can remain in filtrate, but this will not lead to noteworthy decrease in yield and purity.
7. After centrifugation, Pour the flow-through from step 6 into Binding Column (yellow color, inserted into the 50 ml disposable tube). And then centrifuge for 10 min at 3,500 rpm. Remove Binding Column, discard the flow-through, and re-insert Binding Column to used 50 ml tube.
8. Add 20 ml of Washing Buffer A and centrifuge for 5 min at 3,500 rpm.
9. Discard flow-through. Add 20 ml of Washing Buffer B and centrifuge for 5 min at 3,500 rpm.
10. To dry the membrane of Binding Column, centrifuge for 20 min at 3,500 rpm. And then transfer the Binding Column to a new 50 ml disposable tube.
    Note : Completely remove ethanol. Residual ethanol from Washing Buffer B may inhibit subsequent enzymatic reaction and other down stream test.
11. Add 2 ml of Elution Buffer and incubate 10 min at room temperature. Centrifuge for 10 min at 3,500 rpm.

PROTOCOL B (Vacuum Protocol)

1. Pick a single colony from a freshly streaked bacterial plate and use it to inoculate LB (+antibiotics). And then Grow at 37 ℃ for 12 ~ 16 hrs with vigorous shaking (OD_{600} = 1.0 ~ 1.5).
   Note : Use the appropriate volume of bacterial cultures; 100 ~ 150 ml for high copy number plasmid, up to 300 ~ 400 ml for low copy number plasmid.
2. Harvest the bacteria culture by centrifugation for 10 min at 13,000 rpm at 4℃.
   Note : Remove all traces of supernatant by inverting the open tube until all media has drained.
3. Resuspend pelleted bacterial cell thoroughly in 6 ml of M1 Buffer by vortexing until no clumps remain.
   Note : Ensure that RNase A Solution has been added to M1 Buffer. It is essential to completely resuspend the cell pellet. It may affect the lysis efficiency.

Continue : PROTOCOL B (Vacuum Protocol)

4. Add 6 ml of M2 Buffer to resuspended cells and mix by inverting the tube 10 times. DO NOT VORTEX and incubate for 3min at RT.
   Note : The optimal lysis time allows maximum release of plasmid DNA without release of chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. Long exposure to alkaline condition may cause the plasmid to become irreversibly denatured. It is important to proceed to next step immediately after the lysate becomes clear without any cloudy clumps. Do not vortex, it may cause shearing of genomic DNA.
5. Add 6 ml of M3 Buffer and gently mix by inverting the tube 10 times.
   Note : After addition of M3 Buffer, the solution should become cloudy and a fluffy white form. Incubation on ice may help precipitate the denatured cell components more efficiently. The precipitated material contains genomic DNA, protein, cell debris, and SDS.
6. Assemble a column stack by placing Pre Column (clear color) into the top of Binding Column(yellow color). Attach the assembled column stack onto a port of the vacuum manifold tightly. And then pour all of the lysate to Pre Column. (See the EXPERIMENTAL INFORMATION's Figure 1.)
7. Apply maximum vacuum, continuing until all liquid has passed through assembled column stack.
8. Slowly release the vacuum from the filtration device. Remove the Pre Column (clear color), leaving the Binding Column (yellow color) on the manifold.
9. Add 20 ml of Washing Buffer A to Binding Column and allow the vacuum to pull the solution through the Binding Column.
10. Add 20 ml of Washing Buffer B to Binding Column and allow the vacuum to pull the solution through the Binding Column.
11. Slowly release the vacuum. And then transfer to new 50 ml disposable tube.
12. To dry the membrane of Binding Column, centrifuge for 20 min at 3,500 rpm. And then transfer the Binding Column to a new 50 ml disposable tube.
    Note :Completely remove ethanol. Residual ethanol from Washing Buffer B may inhibit subsequent enzymatic reaction and other down stream test.
13. Add 2 ml of Elution Buffer and incubate 10 min at room temperature. Centrifuge for 10 min at 3,500 rpm.

EXPERIMENTAL INFORMATION

● *Shape and application of Pre & Binding Columns*

(a)

Binding Column

Pre Column

(b)

(c)

→ Pre Column (Clear color)

→ Binding column (Yellow color)

→ Vacuum manifold

Fig. 1. Shape and application of Pre & Binding Column
(a) Normal shape of Pre-filtration column & Binding column
Yellow color : Binding Column, Clear color : Pre Column
(b) Shape of 50ml tube application (Protocol A)
(c) Shape of vacuum manifold application (Protocol B)

● *Tendency of plasmid DNA yield & purity from plasmid size*

Plasmids up to approximately 40 Kb can be purified using DNA-maxi™ SV Plasmid DNA Purification Kit.

[Table. 1] Yield & purity of various size plasmid DNA isolated from DH5α.

No.	Sample (Size)	Conc.(ng/μl)	Yield (μg)	$A_{260/280}$
1	pUC 18 (2.9 Kb)	135.01	270	1.82
2	pTA (7.1 Kb)	127.66	255.3	1.78
3	pCEP4 (10.5 Kb)	125.35	250.7	1.88
4	pAdEASY-1 (33.2 Kb)	187.00	274.0	1.78
5	pJM17 (40.2 Kb)	90.66	181.2	1.83

● *Tendency of plasmid DNA from various E. coli strains*

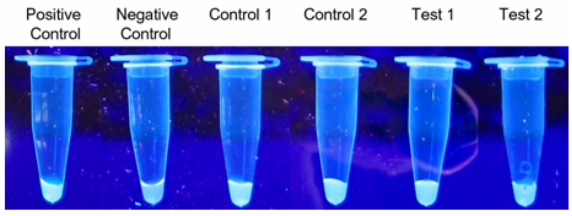
Plasmid DNA(pUC18) were extracted from different kinds of *E. coli* host strains. The recovery showed slightly different yields as host strains but the purity showed high and similar value.

[Table. 2] Yield & purity of plasmid DNA isolated from various *E. coli* host strain

No.	Sample	Conc.(ngl/μ)	Yield (μg)	$A_{260/280}$
1	DH5a	137.32	274.6	1.78
2	JM109	105.42	210.8	1.82
3	TOP10	130.33	260.6	1.79
4	BL21	134.67	269.3	1.89
5	BL21(DE3)	135.33	270.7	1.83
6	BL21(DE3)pLysS	133.00	266.0	1.75

● *in vitro Translation with purified plasmid DNA*

(a)



(b)

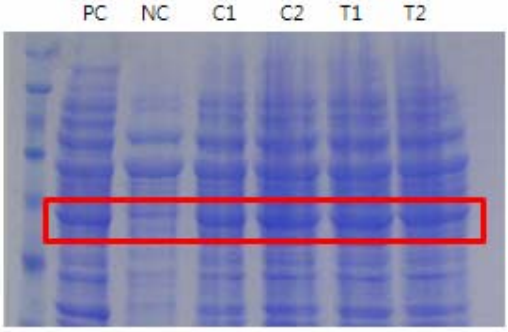


Fig. 2. *in vitro* translation experiment with Genelator™ *in vitro* transcription / Translation Kit (iNtRON, Cat. No. 12011 / 12012)

The purified pEGFP plasmid DNA from DNA-maxi™ SV Plasmid DNA Purification Kit and DNA-spin™ Plasmid DNA Purification Kit (iNtRON, Cat. No. 17093) were applied *in vitro* translation
(a) UV detecion
(b) 12% SDS-PAGE gel running

PC, Positive control (ultra pure pEGFP); NC, Negative control (DW); C1 & C2, Control (DNA-spin™ Plasmid DNA Purification Kit); T1, DNA-midi™ SV Plasmid DNA Purification Kit (iNtRON, Cat.No. 17252); T2, DNA-maxi™ SV Plasmid DNA Purification Kit

•*Agarose gel analysis of remained plasmid DNA at each stage of plasmid DNA purification procedure*

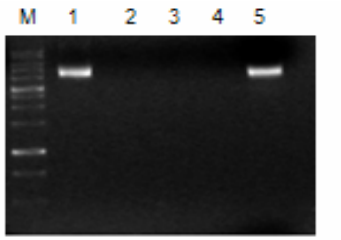


Fig. 3. Agarose gel analysis of plasmid DNA purification protocol.

This test progress due to confirm DNA loss at each stage of plasmid DNA purification procedure. Take sample 1/10 volume of buffer total volume at each stage of plasmid DNA purification procedure, . Plasmid DNA purified by EtOH precipitation.
Lane M, 1 Kb Ladder DNA Marker; lane 1, Pre-filtration flow-thorough; lane 2, Binding flow-Through ; lane 3, Washing A flow through; lane 4, Washing B flow through; lane 5, Final elute.

TROUBLESHOOTING GUIDE

Problem	Possible Causes	Recommendation
Low or no yield of plasmid DNA	Too many cells in sample	Culture should be grown for 12 ~ 16 hours in proper media with antibiotics. Starting sample volume must be reduced.
	M2 Buffer precipitated	Check the M2 Buffer for SDS precipitation due to low storage temperature and dissolve the SDS by warming to 37℃
	Cell resuspension incomplete	Pelleted cells should be completely resuspended in M1 Buffer. Do not add M2 Buffer until an even suspension is obtained.
	M2 Buffer incompletely mixed	Ensure complete mixing all buffers. When put and mix M buffer and M3 Buffer, do not mix strongly
Plasmid DNA degradation	Endonuclease contamination	Because it is different that have endonuclease containing in host strain, consider changing *E*.coli host strain. When put and mix M2 Buffer and M3 Buffer, do not shake strongly
Smearing of plasmid DNA	Too long lysis time	Too long lysis under M2 Buffer can cause chromosomal DNA contamination. Proceed to next step immediately after no more clumps are visible in the lysate.
	Vigorous mixing in M2 buffer	Vigorous handling after addition of M2 Buffer can lead to irreversible denaturation of plasmid DNA. Gentle inverting and rotating tube to cover walls with viscous lysate is sufficient for mixing.

RELATED PRODUCTS

Product Name	Cat. No.
DNA-spin™ Plasmid DNA Purification Kit	17093
DNA-maxi™ SV Plasmid DNA Purification Kit	17253
MEGAquick-spin PCR & Agarose Gel DNA Extraction Kit	17281 / 17282
LINKeed Rapid DNA Ligation Kit (Version 2.0)	15023
CCC (DH5a 1x107, Competent Cell for Cloning)	15045
CCC (DH5a 1x109, Competent Cell for Cloning)	15046
CCC (JM109 1x107, Competent Cell for Cloning)	15047
CCC (JM109 1x109, Competent Cell for Cloning)	15048
CCC (TOP 10 1x107, Competent Cell for Cloning)	15049
CCC (TOP 10 1x109, Competent Cell for Cloning)	15050
Maxime™ PCR PreMix (i-Taq)	25025
Maxime™ PCR PreMix (i-StarTaq)	25165 / 25167
Maxime™ PCR PreMix (i-Pfu)	25185
Maxime™ PCR PreMix (i-MAX II)	25265
Genelator™ *in vitro* Transcription & Translation Kit	12011