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 6 ml dissolved 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
  : *end*A+ 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-maxi™ 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 (Lyophilized powder) 66 mg (33 mg x 2ea)
  : Dissolve the RNase A in 3.3 ml of pure D.W. to each vial. The lyophilized RNase A can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized RNase A can only be dissolved in D.W.; dissolved RNase A should be immediately stored at -20℃. The RNase A solution is stable at -20°C for up to 24 months. Do not refreeze aliquots after thawing.
- Pre Column (Clear Color) 12 Columns
  : Inserted into the 50 ml disposable tube (collection tube).
- Binding Column (Yellow Color) 12Columns
  : Inserted into the 50 ml disposable tube (collection tube).

ADDITIONAL REQUIRED EQUIPMENT

- Absolute EtOH
- Vacuum manifold & vacuum pump
- **Centrifuge : Swinging bucket rotor only**
  : ability of 3,500 ~ 4,000 rpm (2,500 ~ 3,200 g)
- 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 200 ~ 250 ml for low copy number plasmid.
2. Harvest the bacteria culture by centrifugation for 10 min at 3,500 ~ 4,000 rpm (2,500 ~ 3,200 g) and discard supernatant.
   Note : Drain tubes on a paper towel to remove excess media.
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 3 min 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.
   Note : If the M2 buffer becomes too cold, SDS precipitation may occur, leading to poor cell lysis. If a precipitate has formed, warm the M2 buffer to 37°C with gentle shaking.
5. Add 6 ml of M3 Buffer and gently mix by inverting the tube 10 times then incubate the tube in ice for 5 min.
   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 5 min at 3,500 ~ 4,000 rpm (2,500 ~ 3,200 g) .
   Note : A small amount of insoluble material can remain in filtrate, but this will not lead to noteworthy decrease in yield.
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 5 min at 3,500 ~ 4,000 rpm (2,500 ~ 3,200 g). 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 ~ 4,000 rpm (2,500 ~ 3,200 g)
9. Discard flow-through. Add 20 ml of Washing Buffer B and centrifuge for 5 min at 3,500 ~ 4,000 rpm (2,500 ~ 3,200 g)
10. To dry the membrane of Binding Column, centrifuge for 20 min at 3,500 ~ 4,000 rpm (2,500 ~ 3,200 g). 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.
    Note : (Recommended) Place the Binding Column into a new 50 ml collection tube, open the lid, and incubate the assembly at 56 °C for 5 min to dry the membrane completely. This step serves to evaporate any remaining liquid.
11. Add 2 ml of Elution Buffer and incubate 5 min at room temperature. Centrifuge for 5 min at 3,500 ~ 4,000 rpm (2,500 ~ 3,200 g).

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 200 ~ 250 ml for low copy number plasmid.
2. Harvest the bacteria culture by centrifugation for 10 min at 3,500 ~ 4,000 rpm (2,500 ~ 3,200 g) and discard supernatant.
   Note : Drain tubes on a paper towel to remove excess media.
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 3 min 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.
   Note : If the M2 buffer becomes too cold, SDS precipitation may occur, leading to poor cell lysis. If a precipitate has formed, warm the M2 buffer to 37°C with gentle shaking.
5. Add 6 ml of M3 Buffer and gently mix by inverting the tube 10 times then incubate the tube in ice for 5 min.
   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 ~ 4,000 rpm (2,500 ~ 3,200 g). 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.
    Note : (Recommended) Place the Binding Column into a new 50 ml collection tube, open the lid, and incubate the assembly at 56 °C for 5 min to dry the membrane completely. This step serves to evaporate any remaining liquid.
13. Add 2 ml of Elution Buffer and incubate 5 min at room temperature. Centrifuge for 10 min at 3,500 ~ 4,000 rpm (2,500 ~ 3,200 g).

EXPERIMENTAL INFORMATION

• *Shape and application of Pre & Binding Columns*

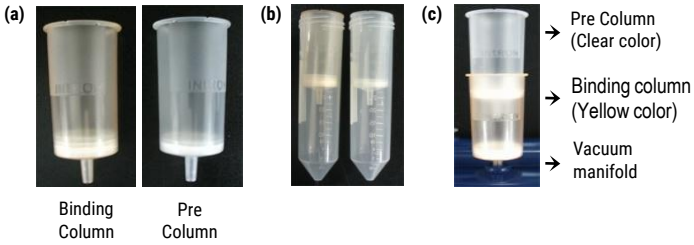

Fig. 1. Shape and application of Pre & Binding Column
(a) Normal shape of Pre-filtration column & Binding column
Yellow color : Binding Column, Clear color : Pre Column
(b) Shape of 50ml tube application (Protocol A)
(c) Shape of vacuum manifold application (Protocol B)

• *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.

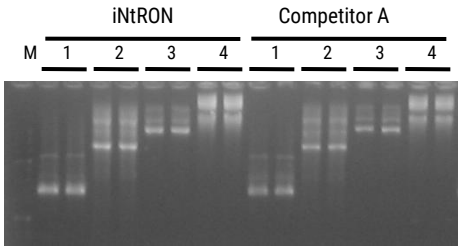

Fig. 2. Agarose gel analysis of plasmid DNA from iNtRON and other companiy kit
Various size of plasmid DNA is purified using DNA-maxi™ SV Kit and other company kit.
Lane M, 1 Kb Ladder DNA Marker; **lane 1**, pUC 18 (2.9kb); **lane 2**, pET (5.8kb); **lane 3**, pCEP4 (10.5kb); **lane 4**, pAdEasy (33.2kb)

Table. 1. Yield & purity of various size of plasmid DNA isolated from *E. coli* (DH5a)

	Vector (Size)	Yield (μg)	$A_{260/280}$	$A_{260/230}$
iNtRON	pUC18 (2 .9 kb)	227	1.82	2.01
	pET (5.8 kb)	173	1.79	1.96
	pCEP4 (10.5 kb)	192	1.83	1.91
	pAdEasy (33.2 kb)	216	1.80	2.05
Competitor A	pUC18 (2.9 kb)	162	1.74	2.31
	pET (5.8 kb)	134	1.79	1.88
	pCEP4 (10.5 kb)	151	1.68	1.96
	pAdEasy (33.2 kb)	133	1.61	1.73

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

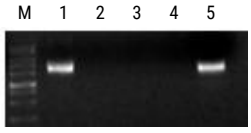

Fig. 3. Agarose gel analysis of plasmid DNA purification protocol.
This test progress due to confirm DNA loss at each stage of plasmid DNA purification procedure.
In order to estimate loss of pDNA, each fraction which was harvested every single step was treated EtOH precipitation.
Lane M, 1 Kb Ladder 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 eluate.

• *Tendency of plasmid DNA yield & purity from start amount*

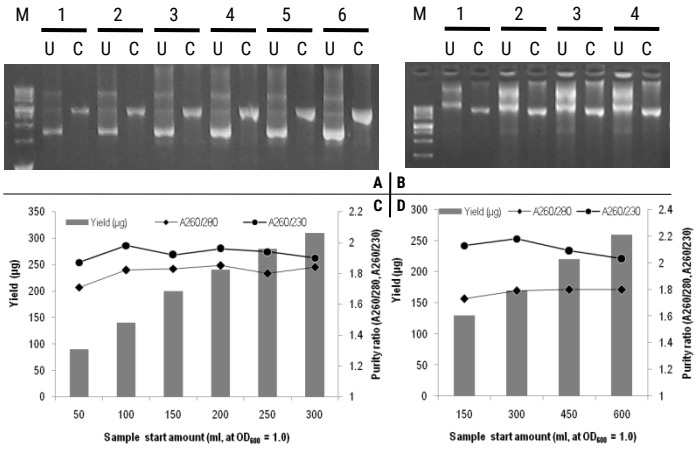

Fig. 4. Influence of sample start amount on pDNA recovery
A : Gel analysis of different amount sample (high copy plasmid; HCP)
Lane M, 1 Kb Ladder DNA Marker; **lane 1**, 50 ml Culture; **lane 2**, 100 ml culture; **lane 3**, 150 ml culture; **lane 4**, 200 ml culture; **lane 5**, 250 ml culture; **lane 6**, 300 ml culture; **lane U,** isolated plasmid DNA; **lane C,** linearized plasmid with *EcoRI*

B : Gel analysis of different amount sample (low copy plasmid; LCP)
Lane M, 1 Kb Ladder DNA Marker; **lane 1**, 150 ml Culture; **lane 2**, 300 ml culture; **lane 3**, 450 ml culture; **lane 4**, 600 ml culture; **lane U,** isolated plasmid DNA; **lane C,** linearized plasmid with *EcoRI*

C : Yield and purity of pDNA from different amount sample (HCP)

D : Yield and purity of pDNA from different amount sample (LCP)

• *Estimation of optimal elution volume*

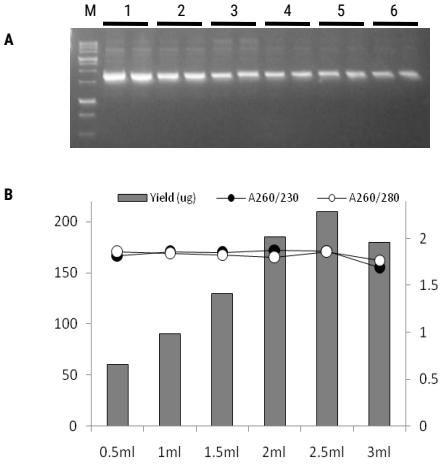

Fig. 5. Influence of elution volume on pDNA recovery (HCP)
A : Gel analysis of different volume of elution buffer
Lane M, 1 Kb Ladder DNA Marker; **lane 1**, 0.5 ml elution; **lane 2**, 1 ml elution; **lane 3**, 1.5 ml elution; **lane 4**, 2 ml elution; **lane 5**, 2.5 ml elution; **lane 6**, 3 ml elution

B : Quality and quantity of pDNA from different volume of elution buffer.

• *Estimation of optimal drying spin time*

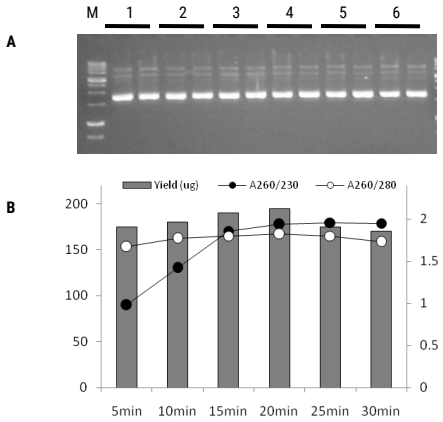

Fig. 6. Influence of time of column drying centrifugation on pDNA recovery (HCP)
In order to evaluate the optimal drying centrifuging time, different spin time was tested from 5 min to 30 min. In result, 20 ~ 25 min was optimal for purity of pDNA recorvery.
A : Gel analysis of different spin time of column drying step
Lane M, 1 Kb Ladder DNA Marker; **lane 1**, 5 min spin; **lane 2**, 10 min spin; **lane 3**, 15 min spin; **lane 4**, 20 min spin; **lane 5**, 25 min spin; **lane 6**, 30 min spin

B. Yield and purity of pDNA from different spin time of column drying step.

• *in vitro Translation with purified plasmid DNA*

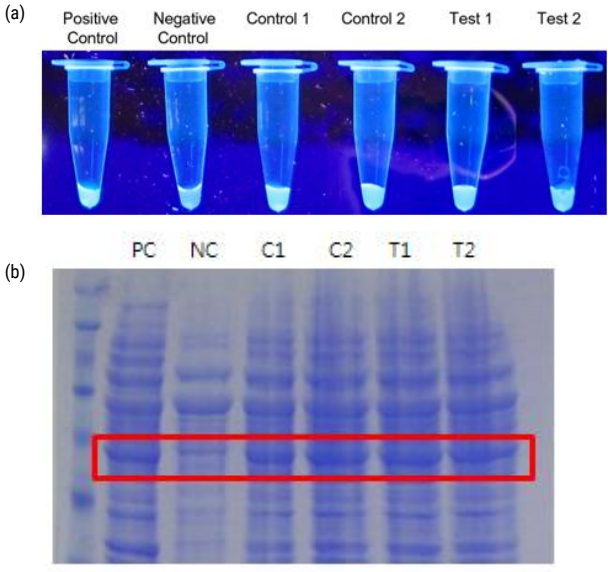

Fig. 7. *in vitro* translation experiment with Genelator™ *in vitro* transcription / Translation Kit (iNtRON, Cat. No. 12011 / 12012)

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 detection
(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

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
	Wrong centrifuge used	The spin protocol of this kit is optimized for swinging bucket rotor. Check the type of centrifuge.
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
Low Purity	Lysate is not clarified through the Pre Column	Keep the ice incubation step after M3 buffer treatment. Un-clear lysate doesn't affect DNA recovery but has an affect the purity of pDNA
	Ethanol carry-over	Ethanol carryover is detected in the final product using the centrifugation method. After the wash step in the centrifugation protocol, transfer the binding column to a new 50 ml tube and repeat centrifugation for 5 minutes to remove residual ethanol. Washing buffer could be present on the outside of the column due to splashing during the wash step. Remove any residual ethanol from the outside of the column prior to elution.
	Genomic DNA contamination	Vortexing or overmixing after addition of the M2 buffer. Do not vortex samples after addition of M2 buffer to prevent shearing of genomic DNA.
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.

ADDITIONAL INFORMATION

Table. 3. *EndA-* and *EndA+* Strains of E. coli.

End A-		*End A+*	
BJ5183	JM109	BL21(DE3)	P2392
DH1	KRX	CJ236	PR700
DH20	MM294	HB101	Q358
DH21	SK1590	JM83	RR1
DH5a	SK1592	JM101	TB1
JM103	SK2267	JM110	TG1
JM105	SRB	LE392	Y1088
JM106	TOP10	MC1061	BMH 71-18
JM107	XLI-blue	NM554	ES1301
JM108	XLO	NM552	

RELATED PRODUCTS

Product Name	Cat. No.
DNA-spin™ Plasmid DNA Purification Kit	17096/17097/17098
DNA-midi™ SV Plasmid DNA Purification Kit	17252
MEGAquick-spin™ Total Fragment DNA Purification Kit	17286 / 17287/17288
LINKeed Rapid DNA Ligation Kit (Version 2.0)	15023
CCC (DH5a 1x10^7, Competent Cell for Cloning)	15045
CCC (DH5a 1x10^9, Competent Cell for Cloning)	15046
CCC (JM109 1x10^7, Competent Cell for Cloning)	15047
CCC (TOP 10 1x10^7, Competent Cell for Cloning)	15049
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