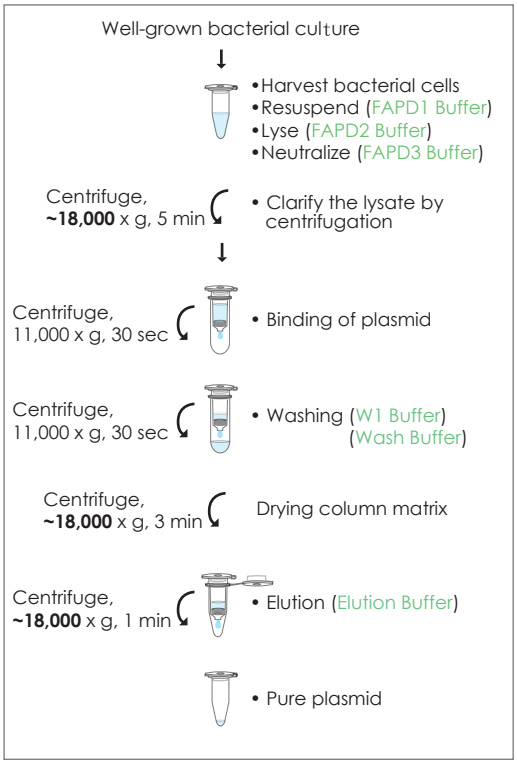
FavorPrep™ Plasmid Extraction Mini Kit

Cat. No.: FAPDE 004
FAPDE 100
FAPDE 300
(For Research Use Only)

Kit Contents:

Cat. No:	FAPDE 004 (4 preps_sample)	FAPDE 100 (100 preps)	FAPDE 300 (300 preps)
FAPD1 Buffer	1.0 ml	25 ml	75 ml
FAPD2 Buffer	1.0 ml	25 ml	75 ml
FAPD3 Buffer	1.5 ml	35 ml	105 ml
W1 Buffer	2.0 ml	45 ml	135 ml
Wash Buffer (concentrate)[a]	1.0 ml	20 ml	50 ml
Elution Buffer	0.5 ml	15 ml	35 ml
FAPD Column	4 pcs	100 pcs	300 pcs
Collection Tube	4 pcs	100 pcs	300 pcs
RNase A	0.1 mg	2.5 mg	7.5 mg
User Manual	1	1	1
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)			
Ethanol volume for Wash Buffer[a]	4 ml	80 ml	200 ml

Brief procedure:



Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1 ~ 3 ml
Size of plasmid or construct:	< 15 kb
Operation time:	< 25 minutes
Typical Yield:	20 ~ 30 µg
Binding capacity:	60 µg/ column
Column applicability:	centrifugation and vaccum

Important Notes:

1. Store RNase A at -20 °C upon recipit of kit.
2. Add 0.5 ml of FAPD1 Buffer to a RNase A tube, vortex the tube to mix well. Briefly spin the tube and transfer the total RNase A mixture back to the FAPD1 bottle, mix well by vortexing and store the FAPD1 buffer at 4 °C.
3. If precipitates have formed in FAPD2 Buffer, warm the buffer in 37°C waterbath to dissolve precipitates.
4. Preparation of Wash Buffer by adding 96 ~100% ethanol (not provided) for first use.
5. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000 ~1,8000 x g.

General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Transfer 1~ 3 ml of well-grown bacterial culture to a centrifuge tube (not provided).
2. Centrifuge the tube at 11,000 x g for 1 minute to pellet the cells and discard the supernatant completely.
3. Add 200 µl of FAPD1 Buffer (RNase A added) to the cell pellet and resuspend the cells completely by pipetting.
- Make sure that RNase A has been added into FAPD1 Buffer when first use.
- No cell pellet should be visible after resuspension of the cells.

4. Add 200 µl of FAPD2 Buffer and gently invert the tube 5 ~ 10 times. Incubate the sample mixture at room temperature for 2 ~ 5 minutes to lyse the cells.
- Do not vortex, vortex may shear genomic DNA. If necessary, continue inverting the tube until the lysate become clear.
- Do not proceed the incubation over 5 minutes.

5. Add 300 µl of FAPD3 Buffer and invert the tube 5 ~ 10 times immediately to neutralize the lysate.
- Invert immediately after adding FAPD3 Buffer will avoid asymmetric precipitation.

6. Centrifuge at full speed (~18,000 x g) for 5 min to clarify the lysate. During centrifugation, place a FAPD Column in a Collection Tube.
7. Transfer the suspernatant carefully to the FAPD Column and centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.
- Do not transfer any white pellet into the column.

8. Add 400 µl of W1 Buffer to the FAPD Column and centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.

9. Add 700 µl of Wash Buffer to the FAPD Column and centrifuge at 11,000 x g for 30 seconds. Discard the flow-through and place the column back to the Collection Tube.

- Make sure that ethanol (96-100 %) has been added into Wash Buffer when first use.

10. Centrifuge at full speed (~ 18,000 x g) for an additional 3 minutes to dry the FAPD Column.

- **Important step !** The residual liquid should be removed thoroughly on this step.

11. Place the FAPD Column to a new 1.5 ml microcentrifuge tube (not provided).

12. Add 50 µl ~ 100 µl of Elution Buffer or ddH2O to the membrane center of the FAPD Column. Stand the column for 1 minute.

- Important step ! For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely.

> • **Note !** Do not Elute the DNA using less than suggested volume (50ul). It will lower the final yield.

13. Centrifuge at full speed (~ 18,000 x g) for 1 minute to elute plasmid DNA and store the DNA at -20 °C.

Troubleshooting

Low yield

Bacterial cells were not lysed completely

- Too many bacterial cells were used (OD600 > 10). Separate the bacterial culture into multiple tubes.
- After FAPD3 Buffer addition, break up the precipitate by inverting to ensure higher yield.

Overgrown of bacterial cells

- Incubation time should not longer than 16 hours.

Bacterial cells were insufficient

- Ensure that bacterial cells have grown to an expected amount (OD600 > 1) after incubation under suitable shaking modes.

Incorrect DNA elution step

- Ensure that Elution Buffer was added and absorbed to the center of the FAPD Column matrix.

Incomplete DNA Elution

- If size of DNA fragments is larger than 10 kb, use preheated Elution Buffer (60~70°C) on slution step to improve the elution efficiency.

Incorrect preparation of Wash Buffer

- Ensure that the correct volume of ethanol (96 ~ 100 %) was added to and Wash Buffer pior to use.

Eluted DNA does not perform well

Residual ethanol contamination

- After Wash Step, dry the FAPD Column with an additional centrifugation at top speed (~18,000 x g) for 5 minutes or incubation at 60°C for 5 minutes.

Genomic DNA Contaminates

Lysate prepared improperly.

- Gently invert the tube after adding the FAPD2 Buffer. And the incubation time should not longer than 5 minutes.
- Do Not use overgrown bacterial culture.

RNA Contaminates Plasmid DNA

Insufficiency of RNase A activity in FAPD1 Buffer because of long-term storage

- Prior to using FAPD1 Buffer, ensure that RNase A was added. If RNase A added FAPD1 Buffer is out of date, add additional RNase A into FAPD1 Buffer to a concentration of 50 µg/ ml then store 4°C.
- Too many bacterial cells were used, reduce sample volume.

Smearing or degrading of Plasmid DNA

Nuclease contamination

- If used host cells have high nuclease activity (e.g., *enA*$^+$ strains), perform the following optional Wash Step to remove residuary nuclease.
  a. After DNA Binding Step, add 400 µl of W1 Buffer into the FAPD Column and incubate for 2 minutes at room temperature.
  b. Centrifuge at full speed (~18,000 xg) for 30 seconds.
  c. Proceed to step 9.

Plasmid DNA is not adequate for enzymatic digestions

Eluted plasmid DNA contains residual ethanol

- Make sure you have discarded the flow-through after washing with Wash Buffer (Step 9) and centrifuged for an addition 3 minutes (Step 10).

Denatured Plasmid DNA migrate faster than supercoilded form during electrophoresis

Incubation in FAPD2 Buffer too long

- Do not incubate the sample longer than 5 minute in FAPD2 Buffer

FavorFilter™ Plasmid Midi SP Kit

(For Research Use Only)

Kit Contents:

Cat No.	FAPDE005-M-002 (2 preps)	FAPDE005-M-025 (25 preps)	FAPDE005-M-050 (50 preps)
PDEA Buffer	3 ml	40 ml	80 ml
PDEB Buffer	3 ml	40 ml	80 ml
PDEC Buffer	3 ml	40 ml	80 ml
W1 Buffer (concentrate)	1.8 ml *	24 ml *	48 ml *
Wash Buffer (concentrate)	2.5 ml **	25 ml **	50 ml **
Elution Buffer	2 ml	25 ml	50 ml
RNase A	0.3 mg	4 mg	8 mg
Midi Binding Column	2 pcs	25 pcs	50 pcs
5 ml Filter Syringe	2 pcs	25 pcs	50 pcs
15 ml tubes	4 pcs	50 pcs	100 pcs

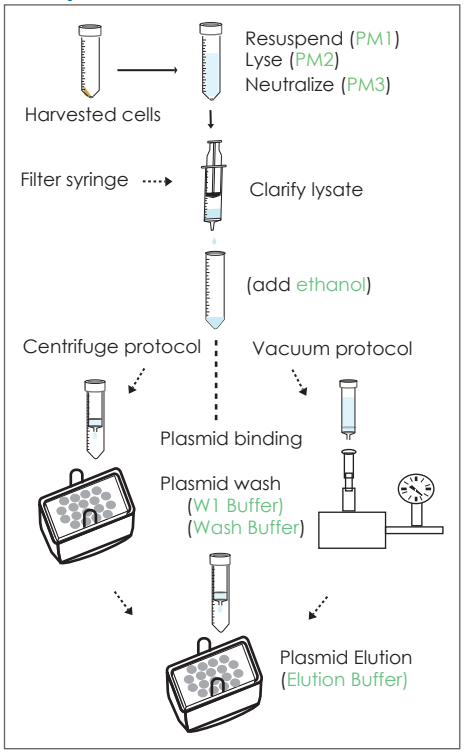
* & ** Add the indicated volume of 100 % ethanol to concentrated W1 Buffer and Wash Buffer.

Cat No.	FAPDE005-M-002	FAPDE005-M-025	FAPDE005-M-050
* W1 Buffer (concentrate)	Add 0.6 ml Ethanol	Add 8 ml Ethanol	Add 16 ml Ethanol
** Wash Buffer (concentrate)	Add 10 ml Ethanol	Add 100 ml Ethanol	Add 200 ml Ethanol

Specification:

Principle:	spin column (silica matrix)
Sample size:	up to 15 ml cell culture
Operation time:	< 60 minutes
Binding capacity:	up to 250 µg/ column
Column applicability:	centrifugation and vaccum

Brief procedure:



Materials and equipmentsupplied by user:

1. Pipettes and pipette tips
2. vortex
3. 100% ethanol
4. Swing bucket centrifuge for 15 ml centrifuge tube (4,500 ~ 6,000 x g) for centrifuge protocol
5. Vacuum manifold for vacuum protocol

Important Notes:

1. Store RNase A at -20 °C upon recipit of kit.
2. Add 0.5 ml of PDEA Buffer to the RNase A tube, vortex the tube to dissolve the RNase A well. Transfer the total RNase A mixture back to the PDEA bottle. Mix well by vortexing and store the PDEA buffer at 4 °C.
3. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffers.
4. Check PDEB Buffer before use to see if any precipitate formed, If yes, warm Buffer PEDB in a 37 °C waterbath to dissolve precipitates.
5. To avoid acidification of PDEB Buffer from CO_2 in the air. Close the bottle immediately after use.
6. For centrifuge protocol, the centrifuge rotor for 15 ml tube should be swig-bucket type to make the plasmid bind symmetrically to the membranes.

Centrifuge Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Harvest the cells from up to 15 ml of bacterial culture by centrifugation at 4,500 ~ 6,000 x g for 10 min.
   Note: Do not use the culture volume more than 15 ml.
2. Add 0.8 ml of PDEA Buffer (RNase A added) to resuspend the cell well by vortexing or pipetting.
3. Add 0.8 ml of PDEB Buffer and mix by inverting the tube 10 times.
   Note: Do not vortex to avoid librating chromosomal DNA into the lysate.
4. Incubate the sample mixture for 5 min at room temperature until lysate becomes slightly clear.
   Note: Do not incubate the sample more than 5 min.
5. Add 0.8 ml of PDEC Buffer and mix immediately by inverting the tube 10 times or more to make the lysate mix well.
   Note: The viscous will not be seen after the lysate mix completely.
6. Put a barrel of 5 ml Filter Syringe on a 15 ml tube and pour the lysate from step 5 into the barrel of Filter Syringe.
7. Incubate the lysate in the barrel for 5 min at room temperature.
   Note: To ensures filtration without clogging, 5 min incubation is required to make the precipitate float up.
8. Gently insert the plunger into the barrel of Filter Syringe and press the plunger to filter the lysate into the 15 ml tube until the air inside the barrel be released. Discard the Filter syringe.
   Note: Do not press the cell debris hardly after the air inside the barrel is released.

9. Add 2.4 ml of 100% ethanol to the filtrate from step 8. Close the tube cap and mix the sample mixture well by plus-vortexing.
10. Place a Midi Binding Column into a new 15 ml tube. Transfer total of the sample mixture (ethanol added) to the column . Close the cap and centrifuge at 4,500 x g for 2 min. Discard the flow-through and place the column back to the 15 ml tube.
    Note: The centrifuge rotor of 15 ml tube should be swig-bucket type to make plasmid bind symmetrically to the membranes.
11. Wash the column by adding 1 ml of W1 Buffer (ethanol added) to the Midi Binding Column. Close the cap and centrifuge at 4,500 x g for 2 min. Discard the flow-through and place the column back to the 15 ml centrifuge tube.
12. Wash the column by adding 4 ml of Wash Buffer (ethanol added) to the Midi Binding Column. Close the tube cap and centrifuge at 4,500 x g for 2 min. Discard the flow-through and place the column back to the 15 ml centrifuge tube.
13. Repeat Step 12 for noe more washing with Wash Buffer.
14. Centrifuge the tube at 4,500 ~6,000 x g for 10 min.
    Note: The 10 min centrifugation should be done for removing the Wash Buffer from membranes.
15. Carefully transfer the Mid Binding Column to a new 15 ml centrifuge tube (provided). Stand the column for 5 min at room temperature.
    Note: Do not let the tip of the Midi Binding Column touch the flow-through when transfer the column.
    To ensures without ethanol resdiual, 5 min incubation is essential to make the ethanol evaporate.
    Note: 5 min incubation is required to make the ethanol evaporate.
16. Add 0.25 ~ 0.5 ml of Elution Buffer or ddH_2O (pH 7.0- 8.5) to the membranes center of the Midi Binding Column. Stand the column for 2 min at room temperature.
    Note: Do not using Elution Buffer or ddH_2O less than suggested volume (0.25 ml). It will lower the final yield.
    Note: For effective elution, stand the Midi Binding Column for 2 min is required to make sure that Elution Buffer is absorbed completely by column membranes.
17. Centrifuge at 4,500 ~6,000 x g for 2 min to elute Plasmid.
18. Use the purified Plasmid in downstream application or store Plasmid at -20 °C.

Vacuum Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Harvest the cells from up to 15 ml of bacterial culture by centrifugation at 4,500 ~ 6,000 x g for 10 min.
   Note: Do not use the culture volume more than 15 ml.
2. Add 0.8 ml of PDEA Buffer (RNase A added) to resuspend the cell well by vortexing or pipetting.
3. Add 0.8 ml of PDEB Buffer and mix by inverting the tube 10 times.
   Note: Do not vortex to avoid librating chromosomal DNA into the lysate.
4. Incubate the sample mixture for 5 min at room temperature until lysate becomes slightly clear.
   Note: Do not incubate the sample more than 5 min.
5. Add 0.8 ml of PDEC Buffer and mix immediately by inverting the tube 10 times or more to make the lysate mix well.
   Note: The viscous will not be seen after the lysate mix complelely.
6. Put a barrel of 5 ml Filter Syringe on a 15 ml tube and pour the lysate from step 5 into the barrel of Filter Syringe.
7. Incubate the lysate in the barrel for 5 min at room temperature.
   Note: To ensures filtration without clogging, 5 min incubation is required to make the precipitate float up.
8. Gently insert the plunger into the barrel of Filter Syringe and press the plunger to filter the lysate into the 15 ml tube until the air inside the barrel be released. Discard the Filter Syringe.
   Note: Do not press the cell debris hardly after the air inside the barrel is released.
9. Add 2.4 ml of 100% ethanol to the filtrate from step 8. Close the tube cap and mix the sample mixture well by plus-vortexing.
10. Connect a Midi Binding Column on the adaptor of vacuum manifold. Transfer total of the sample mixture (ethanol added) to the column. Apply the vacuum until the sample mixture has passed through the membranes completely.
11. Wash the column by adding 1 ml of W1 Buffer (ethanol added) to the Midi Binding Column. Apply the vacuum until the sample mixture has passed through the membranes completely.
12. Wash the column by adding 4 ml of Wash Buffer (ethanol added) to the Midi Binding Column. Apply the vacuum for 10 min to air dry the column membranes.
13. Repeat Step 12 for one more washing with Wash Buffer.
14. Apply the vacuum for 10 min to air dry the column membranes.
    Note: The 10 min vacuum should be done for removing the wash Buffer from membranes.
15. Place the Midi Binding Column into a new 15 ml tube. Free of the cap and stand the column for 5 min at room temperature.
    Note: Wipe the column tip with paper towel to remove residual liquid before place to a new 15 ml tube.
    To ensures without ethanol resdiual, 5 min incubation is essential to make the ethanol evaporate.
    Note: 5 min incubation is required to make the ethanol evaporate.
16. Add 0.25 ~ 0.5 ml of Elution Buffer or ddH_2O (pH 7.0- 8.5)
    to the membranes center of the Midi Binding Column. Stand the column for 2 min at room temperature.
    Note: Do not using Elution Buffer or ddH_2O less than suggested volume (0.25 ml). It will lower the final yield.
    Note: For effective elution, stand the Midi Binding Column for 2 min is required to make sure that Elution Buffer is absorbed completely by column membranes.
17. Centrifuge at 4,500 ~6,000 x g for 2 min to elute Plasmid.
18. Use the purified Plasmid in downstream application or store Plasmid at -20 °C.

™FavorPrep Plasmid DNA Maxi Kit - Endotoxin Removal

(For Research Use Only)

Kit Contents:

Cat No.	FAPDE005C-002 (2 preps)	FAPDE005C-010 (10 preps)	FAPDE005C-020 (20 preps)
PDEA Buffer	14 ml	70 ml	140 ml
PDEB Buffer	14 ml	70 ml	140 ml
PDEC Buffer	14 ml	70 ml	140 ml
Endotoxin Remove Buffer	1.2 ml	6 ml	12 ml
W1 Buffer (concentrate)	7.5 ml *	36 ml *	72 ml *
Wash Buffer (concentrate)	8 ml **	40 ml **	40 ml ** x 2
Elution Buffer	2 ml	25 ml	50 ml
RNase A	1.4 mg	7 mg	14 mg
Midi Binding Column	2 pcs	10 pcs	20 pcs
15 ml tubes	4 pcs	20 pcs	40 pcs

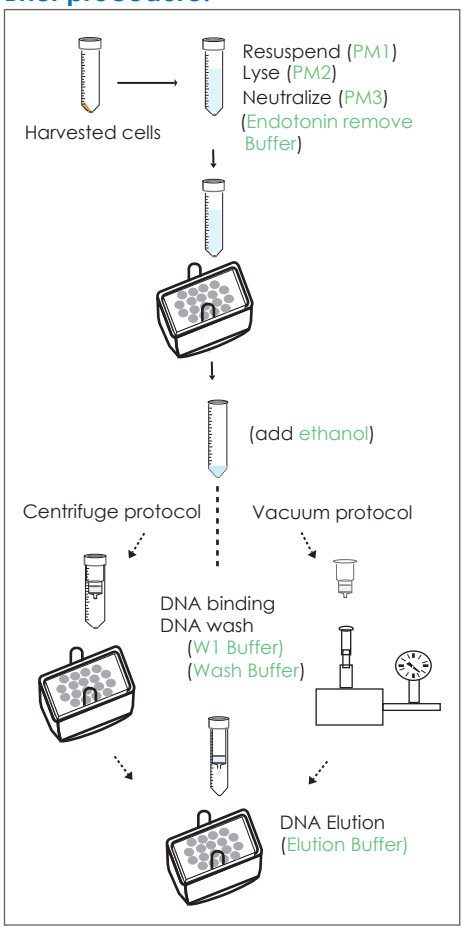
* & ** Add the indicated volume of 100 % ethanol to concentrated W1 Buffer and Wash Buffer.

Cat No.	FAPDE005C-002	FAPDE005C-010	FAPDE005C-020
* W1 Buffer (concentrate)	Add 2.5 ml Ethanol	Add 12 ml Ethanol	Add 24 ml Ethanol
** Wash Buffer (concentrate)	Add 32 ml Ethanol	Add 160 ml Ethanol	Add 160 ml Ethanol

Specification:

Principle:	spin column (silica matrix)
Sample size:	30 ~ 60 ml cell culture
Operation time:	< 60 minutes
Binding capacity:	up to 450 µg/ column
Column applicability:	centrifugation and vaccum

Brief procedure:



Materials and equipmentsupplied by user:

1. Pipettes and pipette tips
2. vortex
3. 100% ethanol
4. Swing bucket centrifuge for 50 ml centrifuge tube (4,500 ~ 6,000 x g) for centrifuge protocol
5. Vacuum manifold for vacuum protocol

Important Notes:

1. Store RNase A at -20 °C upon recipit of kit.
2. Add 0.5 ml of PDEA Buffer to the RNase A tube, vortex the tube to dissolve the RNase A well. Transfer the total RNase A mixture back to the PDEA bottle. Mix well by vortexing and store the PDEA buffer at 4 °C.
3. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffers.
4. Check PDEB Buffer before use to see if any precipitate formed, If yes, Warm Buffer PEDB in a 37 °C waterbath to dissolve precipitates.
5. To avoid acidification of PDEB Buffer from CO_2 in the air. Close the bottle immediately after use.
6. For centrifuge protocol, the centrifuge rotor for 50 ml tube should be swig-bucket type to make the plasmid bind symmetrically to the membranes.

Centrifuge Protocol:

Please Read Important Notes Before Starting Following Steps.

. Harvest the cells from ● 30 ml or ■ 60 ml of bacterial culture by centrifugation at 4,500 ~ 6,000 x g for 10 min.
Note: Do not use the culture volume more than 15 ml.
2. Add ● 2.4 ml or ■ 4.8 ml of PDEA Buffer (RNase A added) to resuspend the cell well by vortexing or pipetting.
3. Add ● 2.4 ml or ■ 4.8 ml of PDEB Buffer and mix by inverting the tube 10 times.
Note: Do not vortex to avoid librating chromosomal DNA into the lysate.
4. Incubate the sample mixture for 5 min at room temperature until lysate becomes slightly clear.
Note: Do not incubate the sample more than 5 min.
5. Add ● 2.4 ml or ■ 4.8 ml of PDEC Buffer and mix immediately by inverting the tube 10 times or more to make the lysate mix well.
Note: The viscous will not be seen after the lysate mix completely.

6. Add ● 0.6 ml or ■ 1.2 ml of Endotoxin Remove Buffer and mix by inverting the tube 10 times. Incubate the sample mixture for 5 min at room temperature.
7. Close the cap and centrifuge at 4,500 ~6,000 x g for 30 min.
8. Transfer ● 7 ml or ■ 14 ml of the supernatant to a new 50 ml centrifuge tube.
9. Add 1 volume (● 7 ml or ■ 14 ml)of 100% ethanol. Close the tube cap, mix the sample mixture well by plus -vortexing.
10. Transfer ● total or ■ half of the sample mixture (ethanol added) to the column . Close the cap and centrifuge at 4,500 x g for 2 min. Discard the flow-through and place the column back to the 50 ml tube.
11. Repeat Step 10 for ■ half of the sample mixture.
    Note: The centrifuge rotor of 50 ml tube should be swig-bucket type to make plasmid bind symmetrically to the membranes.
12. Wash the column by adding 4 ml of W1 Buffer (ethanol added) to the Maxi Binding Column. Close the cap and centrifuge at 4,500 x g for 2 min. Discard the flow-through and place the column back to the 50 ml centrifuge tube.
13. Wash the column by adding 8 ml of Wash Buffer (ethanol added) to the Maxi Binding Column. Close the tube cap and centrifuge at 4,500 ~6,000 x g for 2 min.
14. Repeat Step 13 for one more washing with Wash Buffer.
15. Centrifuge the column at 4,500 ~6,000 x g for 10 min.
    Note: The 10 min centrifugation should be done for removing the Wash Buffer from membranes.
16. Discard the flow-through and carefully transfer the Maxi Binding Column to a new 50 ml centrifuge tube (provided). Free of the cap and stand the column for 5 min at room temperature.
    Note: Do not let the tip of the Maxi Binding Column touch the flow-through when transferring the column.
    Note: 5 min incubation is required to make the ethanol evaporate.
17. Add 0.5 ~1 ml of Elution Buffer or ddH_2O (pH 7.0- 8.5) to the membranes center of the Midi Binding Column. Stand the column for 2 min at room temperature.
    Note: Do not using Elution Buffer or ddH_2O less than suggested volume (0.5 ml). It will lower the final yield.
    Note: For effective elution, stand the Midi Binding Column for 2 min is required to make sure that Elution Buffer is absorbed completely by column membranes.
18. Close the cap and centrifuge at 4,500 ~6,000 x g for 2 min to elute Plasmid.
19. Use the purified Plasmid in downstream application or store Plasmid at -20 °C.

Vacuum Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Harvest the cells from ● 30 ml or ■ 60 ml of bacterial culture by centrifugation at 4,500 ~ 6,000 x g for 10 min.
   Note: Do not use the culture volume more than 15 ml.
2. Add ● 2.4 ml or ■ 4.8 ml of PDEA Buffer (RNase A added) to resuspend the cell well by vortexing or pipetting.
3. Add ● 2.4 ml or ■ 4.8 ml of PDEB Buffer and mix by inverting the tube 10 times.
   Note: Do not vortex to avoid librating chromosomal DNA into the lysate.
4. Incubate the sample mixture for 5 min at room temperature until lysate becomes slightly clear.
   Note: Do not incubate the sample more than 5 min.
5. Add ● 2.4 ml or ■ 4.8 ml of PDEC Buffer and mix immediately by inverting the tube 10 times or more to make the lysate mix well.
   Note: The viscous will not be seen after the lysate mix complelely.
6. Add ● 0.6 ml or ■ 1.2 ml of Endotoxin Remove Buffer and mix by inverting the tube 10 times. Incubate the sample mixture for 5 min at room temperature.
7. Close the cap and centrifuge at 4,500 ~6,000 x g for 30 min.
8. Transfer ● 7 ml or ■ 14 ml of the supernatant to a new 50 ml centrifuge tube.
9. Add 1 volume (● 7 ml or ■ 14 ml)of 100% ethanol. Close the tube cap, mix the sample mixture well by plus -vortexing.
10. Connect a Maxi Binding Column's tip to a adaptor of vacuum manifold. Transfer ● total or ■ half of the sample mixture (ethanol added) to the column . Apply the vacuum until the sample mixture has passed through the membranes completely.
11. Repeat Step 10 for ■ half of the sample mixture.
12. Wash the column by adding 4 ml of W1 Buffer (ethanol added) to the Maxi Binding Column. Apply the vacuum until the sample mixture has passed through the membranes completely.
13. Wash the column by adding 8 ml of Wash Buffer (ethanol added) to the Maxi Binding Column. Apply the vacuum until the sample mixture has passed through the membranes completely.
14. Repeat Step 13 for one more washing with Wash Buffer.
15. Apply the vacuum for 10 min.
    Note: The 10 min vacuum should be done for removing the wash Buffer from membranes.
16. Place the Binding Maxi Column to a 50 ml tube (provided). Stand the column for 5 min at room temperature.
    Note: Wipe the column tip with paper towel to remove residual liquid before place to a new 50 ml tube.
    Note: 5 min incubation is required to make the ethanol evaporate.
17. Place the Midi Binding Column into a new 50 ml tube. Add 0.5 ~ 1 ml of Elution Buffer or ddH_2O (pH 7.0- 8.5) to the membranes center of the Midi Binding Column. Stand the column for 2 min at room temperature.
    Note: Do not using Elution Buffer or ddH_2O less than suggested volume (0.5 ml). It will lower the final yield.
    Note: For effective elution, stand the Midi Binding Column for 2 min is required to make sure that Elution Buffer is absorbed completely by column membranes.
18. Centrifuge at 4,500 ~ 6,000 x g for 2 min to elute Plasmid DNA.
19. Use the purified Plasmid in downstream application or store Plasmid at -20 °C.