Troubleshooting

Problem	Possible Reasons/ Solution
Low yield	**Bacterial cells were not lysed completely** *Too many bacterial cells were used. If use more than 10 A_{600} units of bacterial culture, separate it into multiple tubes. *After PD 3 Buffer addition, break up the precipitate by inverting to ensure higher yield.
	Incorrect Wash Buffer ChecktoensureEthanolwasaddedtoWashBu erpriortouse.
	Incorrect DNA Elution Step *Ensure that Elution Buffer was added and absorbed to the center of Spin Column matrix
	Incomplete DNA Elution If plasmid DNA are larger than 10 kb, use preheat Elution Buffer (60-70°C) on Elution Step to improve the elution efficiency.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** *After wash step, dry Spin Column with additional centrifugation at top speed for 5 minutes or incubation at 60 °C for 5 minutes.
	RNA contamination *Before use PD1 Buffer, check that RNase A was added. If RNase A added PD1 Buffer is overdue, add additional RNase A. *Too many bacterial cells were used, reduce sample volume.
	Genomic DNA contamination *Do not use overgrown bacterial culture. *During PD2 and PD3 Buffer addition, mix gently to prevent genomic DNA shearing.
	Nuclease contamination If host cells have high nuclease activity(e.g.,endA+ strains),perform this Optional Wash Step to remove residual nuclease. Centrifuge at 6000xg (8,000rpm) for 30 seconds. Continue from standard Wash Step.

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BIOMAN SCIENTIFIC CO LTD

EasyPure
Plasmid DNA miniprep kit

Sample:	**1-4 ml of bacterial culture**
Yield:	**up to 30 ug of plasmid/ cosmid DNA**
Format:	**spin column**
Operation time:	**20 min.**
Elution volume:	**50-100 ul**
Storage :	**at room temperature (15-25°C).**

For research use only.
Not for use in diagnostic or therapeutic procedures

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Cat.No. PM100	Cat.No. PM300
100 mini preps / kit	300 mini preps / kit
PD1 Buffer: 25ml	PD1 Buffer: 65ml
PD2 Buffer: 25ml	PD2 Buffer: 75ml
PD3 Buffer: 40ml	PD3 Buffer: 100ml
W1 Buffer: 50ml	W1 Buffer: 130ml
Wash Buffer (concentrated): 25ml*	Wash Buffer (concentrated): 40ml**
Elution Buffer: 6ml	Elution Buffer: 30ml
RNase A (50mg/ml): 50ul	RNase A (50mg/ml): 130ul
2ml Collection Tube: 100pcs	2ml Collection Tube: 300pcs
PD Column: 100pcs	PD Column: 300pcs

* Add 100ml ethanol (96-100%) to Wash Buffer prior to initial use.
** Add 160ml ethanol (96-100%) to Wash Buffer prior to initial use.
Add provided RNase A to PD1 Buffer and store at 4 ^{o}C.
If precipitates have formed in PD2 Buffer , warm the buffer in a 37 ^{o}C water bath to dissolve .
PD3 buffer contains guanidine hydrochloride which is harmful and and irritant. During operation, always wear a lab coat, disposable gloves and protective goggles.

High Copy Number Protocol

Harvesting
1. Transfer 1.5ml of bacterial culture to a microcentrifuge tube (not provided).
2. Centrifuge for 1 min at full speed (13,000 rpm) in a microcentrifuge and discard supernatant.
(If more than1.5 ml of bacteria culture is used, repeat the Harvesting step. For over 4ml, use multiple columns.)

Resuspension
3. Add 200ul of PD1 Buffer (RNase A added) and resuspend the cell pellet by vortexing or pipetting.

Lysis
4. Add 200ul of PD2 Buffer and mix gently by inverting the tube 10 times. Do not vortex, avoid shearing genomic DNA.
5. Allow mixture to stand for 2 minutes at room temperature until lysate clears.

Neutralization
6. Add 300ul of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex.
7. Centrifuge for 2 min at full speed.

DNABinding
8. Place a PD Column in a 2ml Collection Tube.
9. Apply the clear lysate (supernatant) from step 7 to the PD Column.
10. Centrifuge at full speed for 30 seconds.
11. Discard the flow-through and return the PD Column to the 2ml Collection Tube.

Wash
12. Add 400ul of W1 Buffer in the PD Column.
13. Centrifuge at full speed for 30 seconds.
14. Discard the flow-through and return the PD Column to the 2ml Collection Tube.
15. Add 600ul of Wash Buffer (ethanol added) to PD Column.
16. Centrifuge at full speed for 30 Seconds.
17. Discard the flow-through and return the PD Column to the 2ml Collection Tube.
18. Centrifuge again for 3 min at full speed to dry the column matrix.

DNA Elution
19. Transfer the dried PD Column to a clean 1.5ml microcentrifuge tube (not provided).
20. Add 50ul of Elution Buffer or ddH_2O (pH 8.0-8.5) directly onto the centre of the membrane. Avoid residual buffer adhering to the wall of the column.
21. Allow to stand for 2 min until the liquid is absorbed.
22. Centrifuge for 2 min at full speed to elute purified DNA.

Low Copy Number Protocol

Add ethanol and RNase A to buffers according to component instructions .
The typical yield is about 0.5 - 1.0ug per 1 ml culture when preparing low - copy- number plasmid from over night bacterial culture in LB medium .

Harvesting
1. Harvest ep to 10ml of overnight culture by centrifugation.

Resuspension
2. Add 400ul of PD1 Buffer (RNase A added) and resuspend the cell pellet by vortexing or pipetting.

Lysis
3. Add 400ul of PD2 Buffer and mix gently by inverting the tube 10 times. Do not vortex, avoid shearing genomic DNA.
4. Allow mixture to stand for 2 minutes at room temperature until lysate clears.

Neutralization
5. Add 600ul of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex.
6. Centrifuge for 2 min at full speed.

DNABinding
7. Place a PD Column in a 2ml Collection Tube.
8. Apply 750ul of the clear lysate (supernatant) from step 6 to the PD Column.
9. Centrifuge at 10,000xg (13,000rpm) for 30 seconds. Discard the flow-through and return the PD Column to the 2ml Collection Tube.
10.Apply the remaining clear lysate to the same PD column.
11.Centrifuge at 10,000xg (13,000rpm) for 30 seconds.
12. Discard the flow-through and return the PD Column to the 2ml Collection Tube.

Wash
13. Add 400ul of W1 Buffer in the PD Column.
14. Centrifuge at 10,000xg (13,000rpm) for 30 seconds.
15. Discard the flow-through and return the PD Column to the 2ml Collection Tube.
16. Add 600ul of Wash Buffer (ethanol added) to PD Column.
17. Centrifuge at 10,000xg (13,000rpm) for 30 Seconds.
18. Discard the flow-through and return the PD Column to the 2ml Collection Tube.
19. Centrifuge again for 2 min at full speed to dry the column matrix.

DNA Elution
20. Transfer the dried PD Column to a clean 1.5ml microcentrifuge tube (not provided).
21. Add 50ul of Elution Buffer or ddH_2O (pH 8.0-8.5) directly onto the centre of the membrane.
If plasmid DNA are larger than 10 kb, use preheat Elution Buffer (60-70^{o}C) on Elution Step to improve the elution efficiency.
21. Allow to stand for 2 min until the liquid is absorbed by the matrix.
22. Centrifuge for 2 min at full speed to elute purified DNA.