ATP Biotech Inc.

ATP™ Plasmid Mini Kit
Catalog No. APD100/APD300

ATP™ Plasmid 96-Well Kit
Catalog No. APD9602/ APD9604/ APD9610

ATP™ Plasmid Mini Kit

Store at room temperature (15~25℃)

Contents

Introduction

Format : Spin column
Sample : 1~4 ml bacterial culture
Operation : Centrifuge / vacuum manifold
Expected yield : 25~35 μg per high-copy plasmid ; 5~15 μg per low-copy plasmid
Elution volume : 50~100 μl
Application : DNA Library Screening and Analysis ; DNA Sequencing ; Transformation ; PCR ; Restriction Digestion

ATP™ Plasmid Mini Kit is designed for the rapid, easily-handling, and cost-effective isolation of plasmid or cosmid DNA from 1~4 ml of bacterial cultures. This procedure uses modified method of alkaline lysis (1) and RNase treatment for creating cleared cell lysate with minimal genomic DNA and RNA contaminants. Subsequently, the lysate is neutralized and adjusted to high-salt binding conditions adaptable to the adsorption of DNA in one step. In the presence of a chaotropic salt, the plasmid DNA in the lysate binds to the uniquely designed glass-fiber matrix in the spin column (2). Whereas RNA, cellular proteins, and other unwanted impurities flow through the column and are easily and efficiently removed from reaction mixture. After a brief Washing Step with ethanol-contained Wash Buffer to remove endonucleases, salts and other contaminants, the purified plasmid DNA is eluted by low-salt Elution Buffer or water. The entire procedure can be completed in 20 minutes and the purified plasmid DNA is immediately ready for restriction digestion, ligation, PCR, and sequencing. The procedure does not require DNA phenol extraction and alcohol precipitation.

Quality Control

The quality of ATP™ Plasmid Mini Kit was tested on a lot-to-lot basis. The Kits were tested by isolation of plasmid DNA from 4 ml culture of *E.coli* DH5α transformed with the plasmid pBluescript (A600 >2 units/ml). More than 25 μg of plasmid DNA could be quantified by spectrophotometer. 1 μg of the purified plasmid was used on restriction enzyme digestion with *EcoRI*, and digested DNA is checked by agarose gel analysis.

Kit Contents : Cat.No. / Kit Contents

APD100 (100 preps/kit)	APD300 (300 preps/kit)
PD1 Buffer* : 25 ml	PD1 Buffer* : 65 ml
PD2 Buffer** : 25 ml	PD2 Buffer** : 75 ml
PD3 Buffer : 45 ml	PD3 Buffer : 100 ml
W1 Buffer : 45 ml	W1 Buffer : 130 ml
Wash Buffer (concentrated)*** : 25 ml	Wash Buffer (concentrated)*** : 50 ml
Elution Buffer : 6 ml	Elution Buffer : 30 ml
RNase A (50mg/ml) : 50 μl	RNase A (50mg/ml) : 130 μl
PD Columns : 100 pcs (red/white filter)	PD Columns : 300 pcs (red/white filter)
Collection Tubes : 100 pcs	Collection Tubes : 300 pcs

* Add provided RNase A to PD1 Buffer and store at 4 ℃
** If precipitates have formed in PD2 Buffer, warm the buffer in a 37 ℃ waterbath to dissolve precipitates.
*** Add 100 ml/200 ml ethanol (96~100 %) to Wash Buffer prior to initial use.

Use limitation : For research use only; not for diagnostic or medical purposes

Cautions : PD3 Buffer contains guanidine hydrochloride which is a harmful and irritant agent.
During operation, always wear a lab coat, disposable gloves, and protective goggles.
For more information, please refer to the appropriate material safety data sheets (MSDS).

Recommendation

- For larger volumes of bacterial cultures, ATP™ Plasmid Midi/Maxi Kits are recommended.
- For High-throughput samples processing, ATP™ 96-Well Plasmid DNA Kit is recommended.

Equipments and Reagents are provided by User

- ☐ 37 ℃ shaking incubator
- ☐ Inoculaing loop or sterilized toothpick to pick bacterial colonies
- ☐ Culture tubes or flasks
- ☐ LB medium (sterilized) with appropriate selective antibiotic
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Microcentrifuge with rotor for 2 ml tubes
- ☐ Ethanol (96-100%)

- ☐ APD100 : Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
  APD300 : Add 200 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Add provided RNase A to PD1 Buffer and store at 4 ℃

Sample preparations - Growth of bacterial cultures

1. Pick a single colony from a selective plate and inoculate it into 1-5 ml LB medium containing the appropriate selective antibiotic.
2. Incubate at 37 ℃ with vigorous shaking for 12-16 h.
   (We don't recommend that bacterial growth for more than 16 h, because cells may begin to lyse. Plasmid yield would be reduced.)

High Copy Number Protocol

Harvesting

1. Transfer 1.5 ml of bacterial culture into a microcentrifuge tube (provided by user).
2. Centrifuge at full speed (13,000 rpm) for 1 min in a microcentrifuge and discard the supernatant.
   (If more than 1.5 ml of bacterial culture is used, repeat the Harvesting Step.)

Resuspension

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

Lysis

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

Neutralization

6. Add 300 μl of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex.
7. Centrifuge at full speed for 3 minutes.

DNA Binding

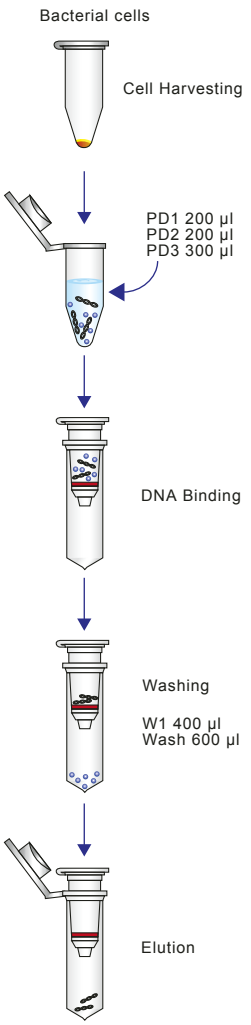
8. Place a PD Column in a Collection Tube.
9. Apply the clear lysate (supernatant) from step 7 into the PD Column.
10. Centrifuge at full speed for 30 seconds.
11. Discard the flow-through and return the PD Column in the 2 ml Collection Tube.

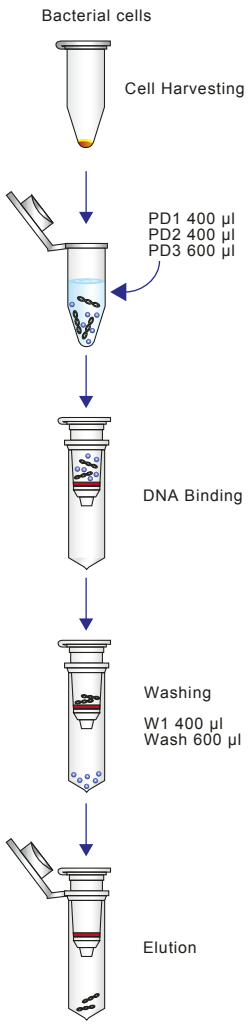
Washing

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

DNA Elution

19. Transfer the dried PD Column in a clean 1.5 ml microcentrifuge tube (provided by user).
20. Add 50 μl of Elution Buffer or ddH_2O (pH 8.0-8.5) directly onto the centre of the membrane.
21. Allow it to stand for 2 min until the liquid is absorbed.
22. Centrifuge at full speed for 2 min to elute purified DNA.





Low Copy Number Protocol

Harvesting

1. Harvest up to 10 ml of overnight culture by centrifuge.

Resuspension

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

Lysis

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

Neutralization

5. Add 600 µl of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex.
6. Centrifuge at full speed for 3 minutes.

DNA Binding

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

Washing

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

DNA Elution

20. Transfer the dried PD Column in a clean 1.5 ml microcentrifuge tube (provided by user).
21. Add 50 µl of Elution Buffer or ddH_2O (pH 8.0-8.5) directly onto the centre of the membrane.
22. Allow to stand for 2 min until the liquid is absorbed.
23. Centrifuge at full speed for 2 min to elute purified DNA.

ATP™ Plasmid 96-Well Kit

Store at room temperature (15~25℃)

Introduction

Format ： 96-well plate
Sample ： 1~2 ml bacterial cultures
Expectant yield ： 5~10 µg per high-copy plasmid；0.5~5 µg per low-copy plasmid
Elution volume ： 50~100 µl
Application ： DNA Library Screening and Analysis；DNA Sequencing；Transformation；PCR；Restriction Digestion

ATP™ Plasmid 96-Well Kit is designed for rapid isolation of plasmid or cosmid DNA from 1-2 ml of bacterial cultures. In the process, the modified method of alkaline lysis (1) and RNase A treatment are used to get cleared cell lysate with minimal genomic DNA and RNA contaminants. In the presence of a chaotropic salt, the plasmid DNA in the lysate binds to glass-fiber matrix in the 96-Well Plasmid Plate (2). The contaminants are washed with an ethanol-contained Wash Buffer, and finally the purified plasmid DNA is eluted by low salt Elution Buffer or water. The protocol does not require DNA phenol extraction and alcohol precipitation.

Quality Control

The quality of 96-Well Plasmid Kit was tested on a lot-to-lot basis. The Kits were tested by isolation of plasmid DNA from 1.5 ml culture of *E.coli* DH5α, which contains the plasmid pBluescript (A600 > 2 units/ ml). More than 8 µg of plasmid DNA could be quantified by spectrophotometer. 1 µg of the purified plasmid was used on restriction enzyme digestion with *EcoRI*, and digested DNA is checked by agarose gel analysis.

Kit Contents：Cat.No. / Kit Contents

APD9602 (2 x 96 prep/kit)	APD9604 (4 x 96 prep/kit)	APD9610 (10 x 96 prep/kit)
PD1 Buffer* ：25 ml	PD1 Buffer* ：65 ml	PD1 Buffer* ：65 ml X 2
PD2 Buffer** ：25 ml	PD2 Buffer** ：75 ml	PD2 Buffer** ：75 ml X 2
PD3 Buffer ：45 ml	PD3 Buffer ：100 ml	PD3 Buffer ：100 ml X 2
Wash Buffer (concentrated)*** ：25 ml	Wash Buffer (concentrated)*** ：25 ml X 2	Wash Buffer (concentrated)*** ：50 ml X 3
Elution Buffer ：30 ml	Elution Buffer ：60ml	Elution Buffer ：60 ml X 2
RNase A (50 mg/ml) ：50 µl	RNase A (50 mg/ml) ：130 µl	RNase A (50mg/ml) ：130 µl X 2
Plasmid Plate ：2 pcs	Plasmid Plate ：4 pcs	Plasmid Plate ：10 pcs
0.35 ml Collection Plate ：2 pcs	0.35 ml Collection Plate ：4 pcs	0.35 ml Collection Plate ：10 pcs
Adhesive film ：4 sheets	Adhesive film ：8 sheets	Adhesive film ：20 sheets

* Add provided RNase A to PD1 Buffer and store at 4 ℃
** If precipitates have formed in PD2 Buffer, warm the buffer in a 37 ℃ waterbath to dissolve precipitates.
*** add 100 ml/200 ml ethanol (96~100%) to Wash Buffer prior to initial use.

Cautions：PD3 Buffer contain guanidine hydrochloride which is a harmful and irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Use limitation : For research use only; not for diagnostic or medical purposes

Centrifuge Protocol

Additional required：

- ☐ Centrifugation system for 96-well plate
- ☐ 2 ml Deep-Well Collection Plate (for collection)
- ☐ 350 µl V-bottom plate (for storage)

Harvesting

1. Transfer 1.5 ml of bacterial culture into each well of 2 ml Deep-Well Collection Plate.
2. Place in a rotor bucket and centrifuge at 3,500 rpm (or 1000 - 1500 xg) for 5-10 min.
3. Remove the medium

Resuspension

4. Add 100 µl of PD1 Buffer (RNase A added) into each well of the plate and resuspend the cell pellet by pipetting or vortexing.

Lysis

5. Add 100 µl of PD2 Buffer into each well of the plate and mix gently by shaking.
6. Allow mixture to stand for 2 minutes at room temperature until lysate clears.

Neutralization

7. Add 150 µl of PD3 Buffer into each well of the plate and mix immediately by shaking.
8. Centrifuge at 3,500 rpm for 5-10 min in a centrifuge.

DNA Binding

9. Place a Plasmid Plate on a 2 ml Collection Plate.
10. Apply the clear lysate (supernatant) from step 8 into the Plasmid Plate.
11. Place in a rotor bucket and centrifuge at 3,500 rpm for 5-10 min.
12. Discard the flow-through and place the Plasmid Plate back on the 2 ml Deep-Well Collection Plate.

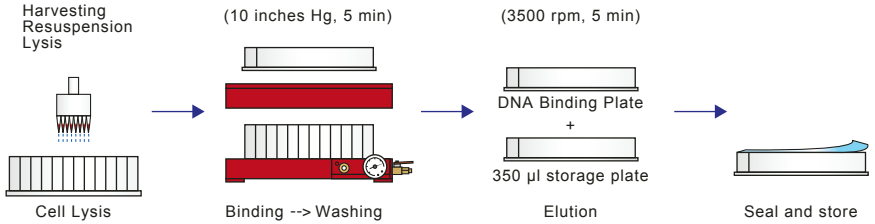
Washing

13. Add 250 µl of Wash Buffer (ethanol added) into each well of the plate.
14. Centrifuge at 3,500 rpm for 5 min in a centrifuge.
15. Add 250 µl of Wash Buffer (ethanol added) into each well of the plate to wash again.
16. Discard the flow-through and place the Plasmid Plate back on the 2 ml Collection Plate.
17. Centrifuge for 10 min at 3,500 rpm in a centrifuge to remove ethanol residue.

DNA Elution

18. Transfer the Plasmid Plate onto a clean 350 µl storage plate.
19. Add 50-100 µl of Elution Buffer or water onto the center of the membrane.
20. Stand for 2 minutes until Elution Buffer or water is absorbed by the matrix.
21. Centrifuge at 3,500 rpm for 5 min in a centrifuge to elute purified DNA.

Vacuum & Centrifuge Protocol



Harvesting

1. Transfer 1.5 ml of bacterial culture into each well of 2 ml Deep-Well Collection Plate.
2. Place in a rotor bucket and centrifuge at 3,500 rpm (or 1000 - 1500 xg) for 5-10 min.
3. Remove the medium

Resuspension

4. Add 100 µl of PD1 Buffer (RNase A added) into each well of the plate and resuspend the cell pellet by pipetting or vortexing.

Lysis

5. Add 100 µl of PD2 Buffer into each well of the plate and mix gently by shaking.
6. Allow mixture to stand for 2 minutes at room temperature until lysate clears.

Neutralization

7. Add 150 µl of PD3 Buffer into each well of the plate and mix immediately by shaking.
8. Centrifuge at 3,500 rpm for 5-10 min in a centrifuge.

DNA Binding

9. Place a Plasmid plate on top of the vacuum manifold.
10. Load the clear lysate (supernatant) from step 8 into the plate (about 350 µl).
11. Apply vacuum at 10 inches Hg for 5 minutes until all wells are empty.

Washing

12. Add 250 µl of Wash Buffer (ethanol added) into each well of the plate.
13. Apply vacuum at 10 inches Hg for 5 minutes until all wells are empty.
14. Add 250 µl of Wash Buffer (ethanol added) into each well of the plate to wash again.
15. Apply vacuum at 10 inches Hg for 5 minutes until all wells are empty.
16. Apply vacuum for another 10 min (or incubate at 60 ℃ 5-10 min) to remove ethanol residue.

DNA Elution

17. Transfer the Plasmid Plate onto a clean 350 µl storage plate.
18. Add 50-100 µl of Elution Buffer or water onto the center of the membrane.
19. Stand for 2 minutes until Elution Buffer or water is absorbed by the matrix.
20. Centrifuge at 3,500 rpm for 5 min in a centrifuge to elute purified DNA.

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. ・Following PD3 Buffer addition, break up the precipitate by inverting to ensure higher yield.
	Incorrect Wash Buffer ・Ensure that correct volume of Ethanol was added to Wash Buffer prior to use.
	Incorrect DNA Elution Step ・Ensure that Elution Buffer was added adequately and absorbed to the center of PD Column matrix ・If DNA was eluted by water, ensure the pH of water ranged from 7.0 to 8.5.
	Incomplete DNA Elution ・If plasmid DNA was larger than 10 kb, use pre-heated Elution Buffer (60-70 ℃) on Elution Step to improve the elution efficiency.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** ・After washing step, dry PD Column with additional centrifugation at full speed for 5 minutes or incubation at 60 ℃ for 5 minutes.
	RNA contamination ・Prior to using PD1 Buffer, ensure that RNase A was added. If RNase A added PD1 Buffer is out of date, add additional RNase A. ・If 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 Washing Step** to remove residual nuclease. ・After DNA Binding Step, add 400 µl of W1 Buffer into PD column and Incubate for 2 minutes at room temperature. ・Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds. ・Continue from standard **Washing Step**.

References

(1) Bimboim, H. C., and Doly, J. (1979) Nucleic Acid Res. 7,1513
(2) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76,615.

Catalog

Product Name	Package	Cat. No.
ATP™ Plasmid Mini Kit	100/300 prep	APD100/APD300
ATP™ Plasmid Midi Kit (Ultra Pure)	25 prep	API25
ATP™ Plasmid Maxi Kit (Ultra Pure)	10/25 prep	APM10/APM25
ATP™ 96-Well Plasmid Mini Kit	4/10 plates	APD9604/APD9610
ATP™ Plasmid Mini Binding Column	50 pcs	PDC50
ATP™ Plasmid Midi Resin Column	10 pcs	PIC10
ATP™ Plasmid Maxi Resin Column	10 pcs	PMC10
ATP™ Gel/PCR DNA Fragments Extraction Kit	100/300 prep	ADF100/ADF300
ATP™ 96-Well Gel/PCR DAN Extraction Kit	4/10 plates	ADF9604/ADF9610
ATP™ 96-Well SEQ Dye Clean Up Kit	4/10 plates	ADC9604/ADC9610
ATP™ Fragment DNA Binding Column	50 pcs	DFC50
ATP™ Genomic DNA Mini Kit (Blood/Culture Cell/Bacteria)	100/300 preps	AGB100/AGB300
ATP™ Genomic DNA Mini Kit (Tissue)	50/300 preps	AGT050/AGT300
ATP™ Genomic DNA Mini Kit (Plant)	100 preps	AGP100
ATP™ Genomic DNA Maxi Kit (Fresh Blood)	25 prep	AGBM25
ATP™™ Genomic DNA Maxi Kit (Frozen Blood)	25 prep	AGDM25
ATP™ Plant Genomic DNA Maxi Kit	25 prep	AGPM25
ATP™ 96-Well Genomic DNA Kit	4/10 plates	AGB9604/AGB9610
ATP™ Reagent Genomic DNA Kit	For 100ml blood	AGE100
ATP™ Genomic DNA Binding Column	50 pcs	GDC50
ATP™ RNA Mini Kit (Blood/Culture Cell/Bacteria)	50 prep	ARB050
ATP™ RNA Mini Kit (Tissue)	50 prep	ART050
ATP™ RNA Mini Kit (Plant)	50 prep	ARP050
ATP™ Viral Nucleic Acid Mini Kit	50 prep	AVR050
ATP™ 96-Well Viral Nucleic Acid Kit	4/10 plates	AVR9604/AVR9610
ATP™ RNA Maxi Kit	10 prep	ARTM10
ATP™ Plant RNA Maxi Kit	10 prep	ARPM10
ATP™ RNA Binding Column	50 pcs	RBC50
Proteinase K	11 mg/kit	APK000011
RNase A	0.2 ml (50 mg/ml)	ARA500200
RNase A	1.5 ml (50 mg/ml)	ARA501500

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