ATP Biotech Inc.

ATP™ Plasmid DNA Midi Kit
Catalog No. API025

ATP™ Plasmid DNA Maxi Kit
Catalog No. APM010/APM025

ATP™ Plasmid Midi Kit

Store at room temperature (15~25℃)

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Introduction

Sample : 20~50 ml of bacterial culture for high-copy number plasmid
100~200 ml of bacterial culture for low-copy number plasmid
Operation : Gravity-flow
Operation time : 120 minutes
Yield : Up to 200 μg of plamsid
Application : Transfection ; Microinjection ; Sequencing ; Restriction Enzyme Digestion ; *in vitro* Transcription

ATP™ Plasmid Midi Kits use pre-packed resin of anion-exchange column to purify plasmid or cosmid DNA from 20~200 ml bacterial cultures. In the process, the modified method of alkaline lysis (1) and RNase treatment are used for creating cleared cell lysate with minimal genomic DNA and RNA contaminants. By a gravity-flow procedure, the plasmid DNA in crude lysate binds to the anion-exchange resin in the appropriate salt and pH conditions. 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 to wash off contaminants, the purified plasmid DNA is eluted by high-salt buffer and then precipitated by isopropanol for desalting. The entire procedure can be completed in 120 minutes without ultracentrifuges, HPLC or other toxic reagents.

Quality Control

The quality of ATP™ Plasmid Midi Kit was tested on a lot-to-lot basis. The Kits were tested by isolation of plasmid DNA from 50 ml culture of *E.coli* DH5α transformed with the plasmid pBluescript (A600 >2 units/ml). More than 120 μg of plasmid DNA could be quantified by spectro-photometer. 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

API025 (25 preps/kit)

PM1 Buffer* : 110 ml
PM2 Buffer** : 110 ml
PM3 Buffer : 110 ml
PEQ Buffer : 130 ml
PW Buffer (concentrated) : 360 ml
PEL Buffer : 220 ml
RNase A (50 mg/ ml) : 200 μl
Plasmid-Midi Columns : 25 pcs

* Add provided RNase A to PM1 Buffer and store at 4 ℃
** If precipitates have formed in PM2 Buffer, warm the buffer in a 37 ℃ waterbath to dissolve precipitates.

ATP™ Plasmid Midi Kit

Store at room temperature (15~25℃)

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

Cautions : 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).

Equipments and Reagents are provided by User

- ☐ 37 ℃ shaking incubator
- ☐ Inoculaing loop or sterilized toothpick to pick bacterial colonies
- ☐ Culture tubes or flasks
- ☐ Culture vessels or bottles with a volume at least 4 times the volume of the culture
- ☐ Sufficient LB medium (sterilized) containing appropriate selective antibiotic
- ☐ Centrifuge with cooler and rotor for 50 ml tubes
- ☐ Ethanol (96-100%)
- ☐ Ice

Sample preparation - Growth of bacterial cultures

1. Pick a single colony from a selective plate and inoculate it into 3-5 ml LB medium containing the appropriate selective antibiotic. Incubate at 37 ℃ with vigorous shaking for 12-16 h.
2. Dilute the starter culture 1/500 to 1/1000 into selective LB medium.
   Midi :
   For high-copy plasmids, inoculate 25-50 μl starter culture into 25-50 ml selective LB medium.
   For low-copy plasmids, inoculate 100-200 μl starter culture into 100-200 ml selective LB medium.
3. Grow at 37 ℃ with vigorous shaking (approx. 250 rpm) for 12-16 h.
   (We would recommend that the culture should reach a cell density of approximately 3-4 x 10^9 cells per milliliter ; We don't recommend that bacterial growth for more than 16 h, because cells may begin to lyse. Plasmid yield would be reduced.)

Plasmid Midi Kit Protocol

- ☐ Use 25-50 ml of bacterial culture for high-copy-number plasmids or 100-200 ml bacterial culture for low-copy-number plasmids.
- ☐ Add provided RNase A to PM1 Buffer and store at 4 ℃.
- ☐ Additional Requirements : 50 ml centrifuge tube、Isopropanol、75 % ethanol

Cell Harvesting / Column Equilibration

1. Harvest the bacterial culture by centrifugation at 6,000 xg for 15 minutes at 4 ℃.
2. Place a Plasmid-Midi Column in a 50 ml centrifuge tube.
3. Equilibrate a Plasmid-Midi column by applying 5 ml of PEQ Buffer, allow the column to empty by gravity flow.
4. Discard the filtrate.

Resuspension

5. Apply 4 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.

Lysis

6. Add 4 ml of PM2 Buffer and mix gently by inverting the tube 10 times. Do not vortex, to avoid shearing the genomic DNA.
7. Stand for 2 minutes on ice until lysate clears.

Neutralization

8. Add 4 ml of PM3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex.
9. Centrifuge at 15,000 xg for 20 minutes at 4 ℃.

DNA Binding

10. Apply the supernatant from step 9 to equilibrated Plasmid-Midi column and allow it to flow through by gravity flow.
11. Discard the filtrate.

Washing

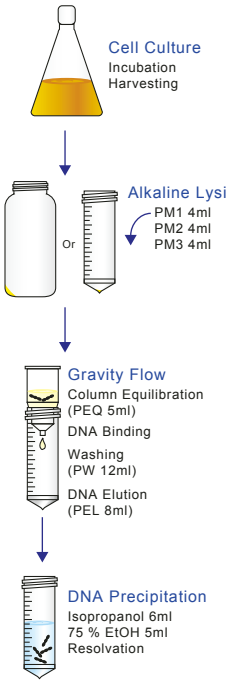
12. Wash the Plasmid-Midi column by applying 12 ml of PW Buffer; allow the column to empty by gravity flow.
13. Discard the filtrate.

Elution

14. Place Plasmid-Midi column in a clean centrifuge tube and add 8 ml of PEL Buffer to elute DNA by gravity flow.

DNA Precipitation

15. Precipitate DNA by adding 6 ml (0.75 volumes) of isopropanol to the eluted DNA from step 14.
16. Mix gently and centrifuge at 20,000 xg for 30 minutes at 4 ℃.
17. Carefully remove the supernatant and wash DNA pellet by 5 ml ethanol(75 %) of room temperature.
18. Centrifuge at 20,000 xg for 10 minutes at 4 ℃.
19. Carefully remove the supernatant and air-dry the DNA pellet for 10 minutes.
20. Dissolve the DNA in 100 μl or a suitable volume of TE or ddH_2O.



Note

Introduction

Sample ： 100~200 ml of bacterial culture for high-copy number plasmid
250~400 ml of bacterial culture for low-copy number plasmid
Operation ： Gravity-flow
Operation time ：120 minutes
Yield ： Up to 500 μg of plamsid
Application ： Transfection；Microinjection；Sequencing；Restriction Enzyme Digestion；*in vitro* Transcription

ATP™ Plasmid Maxi Kits use pre-packed resin of anion-exchange column to purify plasmid or cosmid DNA from 100~400 ml bacterial cultures. In the process, the modified method of alkaline lysis (1) and RNase treatment are used for creating cleared cell lysate with minimal genomic DNA and RNA contaminants. By a gravity-flow procedure, the plasmid DNA in crude lysate binds to the anion-exchange resin in the appropriate salt and pH conditions. 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 to wash off contaminants, the purified plasmid DNA is eluted by high-salt buffer and then precipitated by isopropanol for desalting. The entire procedure can be completed in 120 minutes without ultracentrifuges, HPLC or other toxic reagents.

Quality Control

The quality of ATP™ Plasmid Maxi Kit was tested on a lot-to-lot basis. The Kits were tested by isolation of plasmid DNA from 100 ml culture of *E.coli* DH5α transformed with the plasmid pBluescript (A600 >2 units/ml). More than 400 μg of plasmid DNA could be quantified by spectro-photometer. 1 μg of the purified plasmid was used on restriction enzyme digestion by *EcoRI,* and digested DNA is checked by agarose gel analysis.

Kit Contents：Cat.No. / Kit Contents

APM010 (10 preps/kit)	APM025 (25 preps/kit)
PM1 Buffer* ：110 ml	PM1 Buffer* ：275 ml
PM2 Buffer** ：110 ml	PM2 Buffer** ：275 ml
PM3 Buffer ：110 ml	PM3 Buffer ：275 ml
PEQ Buffer ：130 ml	PEQ Buffer ：275 ml
PW Buffer (concentrated)：360 ml	PW Buffer (concentrated)：240 ml + 550 ml
PEL Buffer：130 ml	PEL Buffer：220 ml + 130 ml
RNase A (50 mg/ ml)：200 μl	RNase A (50 mg/ ml)：550 μl
Plasmid-Maxi Columns：10 pcs	Plasmid-Maxi Columns：25 pcs

* Add provided RNase A to PM1 Buffer and store at 4 ℃
** If precipitates have formed in PM2 Buffer, warm the buffer in a 37 ℃ waterbath to dissolve precipitates.

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

Cautions : 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).

Equipments and Reagents are provided by User

- ☐ 37 ℃ shaking incubator
- ☐ Inoculaing loop or sterilized toothpick to pick bacterial colonies
- ☐ Culture tubes or flasks
- ☐ Culture vessels or bottles with a volume at least 4 times the volume of the culture
- ☐ Sufficient LB medium (sterilized) containing appropriate selective antibiotic
- ☐ Centrifuge with cooler and rotor for 50 ml tubes
- ☐ Ethanol (96-100%)
- ☐ Ice

Sample preparations - Growth of bacterial cultures

1. Pick a single colony from a selective plate and inoculate it into 3-5 ml LB medium containing the appropriate selective antibiotic. Incubate at 37 ℃ with vigorous shaking for 12-16 h.
2. Dilute the starter culture 1/500 to 1/1000 into selective LB medium.
   Maxi :
   For high-copy plasmids, inoculate 100-200 μl starter culture into 100-200 ml selective LB medium.
   For low-copy plasmids, inoculate 250-400 μl starter culture into 250-400 ml selective LB medium.
3. Grow at 37 ℃ with vigorous shaking (approx. 250 rpm) for 12-16 h.
   (We would recommend that the culture should reach a cell density of approximately 3-4 x 10^9 cells per milliliter ; We don't recommend that bacterial growth for more than 16 h, because cells may begin to lyse. Plasmid yield would be reduced.)

Plasmid Maxi Kit Protocol

- ☐ Use 100-200 ml of bacterial culture for high-copy-number plasmids or 250-400 ml bacterial culture for low-copy-number plasmids.
- ☐ Add provided RNase A to PM1 Buffer and store at 4 ℃.
- ☐ Additional Requirements : 50 ml centrifuge tube、Isopropanol、75 % ethanol

Cell Harvesting / Column Equilibration

1. Harvest the bacterial culture by centrifugation at 6,000 xg for 15 minutes at 4 ℃.
2. Place a Plasmid-Maxi Column in a 50 ml centrifuge tube.
3. Equilibrate a Plasmid-Maxi column by applying 10 ml of PEQ Buffer, allow the column to empty by gravity flow.
4. Discard the filtrate.

Resuspension

5. Apply 10 ml of PM1 Buffer (RNase A added) to resuspend the cell pellet by vortexing or pipetting.

Lysis

6. Add 10 ml of PM2 Buffer and mix gently by inverting the tube 10 times. Do not vortex, to avoid shearing the genomic DNA.
7. Stand for 2 minutes on ice until lysate clears.

Neutralization

8. Add 10 ml of PM3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex.
9. Centrifuge at 15,000 xg for 20 minutes at 4 ℃.

DNA Binding

10. Apply the supernatant from step 9 to equilibrated Plasmid-Maxi column and allow it to flow through by gravity flow.
11. Discard the filtrate.

Washing

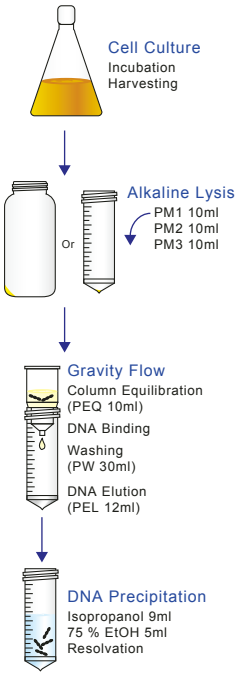
12. Wash the Plasmid-Maxi column by applying 30 ml of PW Buffer; allow the column to empty by gravity flow.
13. Discard the filtrate.

Elution

14. Place Plasmid-Maxi column in a clean centrifuge tube and add 12 ml of PEL Buffer to elute DNA by gravity flow.

DNA Precipitation

15. Precipitate DNA by adding 9 ml (0.75 volumes) of isopropanol to the eluted DNA from step 14.
16. Mix gently and centrifuge at 20,000 xg for 30 minutes at 4 ℃.
17. Carefully remove the supernatant and wash DNA pellet by 5 ml ethanol(75 %) of room temperature.
18. Centrifuge at 20,000 xg for 10 minutes at 4 ℃.
19. Carefully remove the supernatant and air-dry the DNA pellet for 10 minutes.
20. Dissolve the DNA in 300 μl or a suitable volume of TE or ddH_2O.



Troubleshooting

Problem	Possible Reasons / Solution
Low yield	**Bacterial cells were not lysed completely** ・Too many bacterial cells were used. ・Following PM3 Buffer addition, break up the precipitate by inverting to ensure higher yield.
	DNA lose at step17 or step19 ・Remove the supernatant carefully.
Eluted DNA does not perform well in downstream applications	**RNA contamination** ・Prior to using PM1 Buffer, ensure that RNase A was added. If RNase A added PM1 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 PM2 and PM3 Buffer addition, mix gently to prevent genomic DNA shearing

Reference

(1) Bimboim, H. C., and Doly, J. (1979) Nucleic Acid Res. 7,1513

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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