ATP Biotech Inc.

ATP™ Genomic DNA Mini Kit (Plant)
Catalog No. AGP100

ATP™ Genomic DNA Maxi Kit (Plant)
Catalog No. AGPM10

ATP™ Genomic DNA Mini Kit (Plant)

Store at room temperature (15~25℃)

Contents

Introduction

Format ： Spin column
Sample ： 100 mg plant tissue
Operation ： Centrifuge / vacuum manifold
Yield ： 5-30 μg
Elution volume ： 50~200 μl
Application ： PCR、Real-Time PCR、Southern Blotting、AFLP、PADP/ AFLP

ATP™ Plant Genomic DNA Mini Kit provides a fast and simple method to isolate total DNA (genomic DNA, mitochondrial and chloroplast) from plant tissue and cells. In the process, sample is destroyed by grinding in liquid nitrogen and lysis buffer incubation. The Lysate is treated with RNase A to degrade RNA and filtrated by filter column to remove cell debris and salt precipitations. In the presence of binding buffer with chaotropic salt, the genomic DNA in the lysate binds to glass fiber matrix in the spin column (1). The contaminants are washed by wash buffer containing ethanol and finally, the purified genomic DNA is eluted by low-salt elution buffer or water. The protocol does not require DNA phenol extraction and alcohol precipitation. The entire procedure can be completed in 60 minutes.

Quality Control

The quality of ATP™ Genomic DNA Kit (Plant) was tested on a lot-to-lot basis. The Kits were tested by isolation of genomic DNA from 50 mg young leave. The purified DNA was quantified with spectrophotometer and the yield of genomic DNA was more than 10 μg with A260/A280 ratio 1.7 to 1.9.

Kit Contents：Cat.No. / Kit Contents

AGP100 (100 preps/kit)

GP1 Buffer ： 50 ml
GPX1 Buffer： 50 ml
GP2 Buffer ： 15 ml
GP3 Buffer*： 30 ml
W1 Buffer ： 45 ml
Wash Buffer (concentrated)**：25 ml
Elution Buffer：30 ml
RNase A (10mg/ml)：550 μl
Filter Columns：100 pcs
GD Columns：100 pcs (green/white filter)
Collection Tubes：200 pcs

* Add 60 ml isopropanol to GP3 Buffer prior to the initial use.
** Add 100 ml ethanol (96~100 %) to Wash Buffer prior to the initial use.

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

Cautions : The component contains 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).

Expected yield :

Sample	DNA yield (μg)	
	Mini(100mg leaf)	Maxi(1g leaf)
Arabidopsis (Arabidopsis thaliana)	3~5	30~50
Bamboo (Bambusa lodhamii)	10~15	100~150
Camphor tree(Cinnamommun camohora)	15~20	150~200
Chianes Yam (Rhizoma dioscoreae)	30~60	300~500
Maize (Zea mays)	15~20	150~200
Orchis (Phalaenopsis aphrodite)	5~10	50~100
Rice(Oryza sativa)	10~15	100~150
Spinach (Spinacia oleracea)	5~10	50~100
Sweet potato (Ipomoea batata)	20~30	200~300
Tobaco (Nicotiana tabacum)	20~25	200~250
Tomato (Lycopersicon esculentum)	10~15	100~150

Equipments and Reagents are provided by User

- ☐ 1.5 ml microcentrifuge tubes
- ☐ Microcentrifuge with rotor for 2 ml tubes
- ☐ 65 ℃ water-bath or dry-bath
- ☐ Grind tool
- ☐ Liquid nitrogen for sample grinding
- ☐ Ethanol (96-100 %)
- ☐ Ice

Protocol Technical Specifications

Depending on species, plant cells are extremely diverse in their cellular components, such as polysaccharides, lipids, proteins, and other metabolites.
According to this characteristic, we provide two different lysis buffers for best performances with various plant tissues.

Ⅰ. GP1 Buffer is used in the standard protocol for sample lysis. For most of common plant samples, this buffer system is sufficient to ensure purified RNA with high yields and quality.

Ⅱ. There is still an alternative buffer, Buffer GPX1, provided with the kit. The detergent in this buffer system is more effective in handling plant tissues with large quantities of polysaccharides.

Generally, these two buffer systems could achieve adequate performances. Users may try and chose one best for their demands.

Genomic DNA Mini (Plant) Protocol

Tissue Dissociation

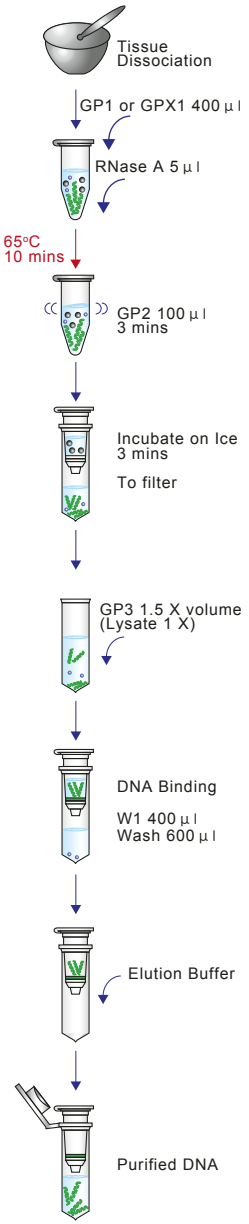
1. Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue or 5 mg (up to 100 mg) of dried sample.
2. Grind the sample under liquid nitrogen to a fine powder. Transfer it into a microcentrifuge tube (provided by user). For some plant sample, user can destruct it without liquid nitrogen.

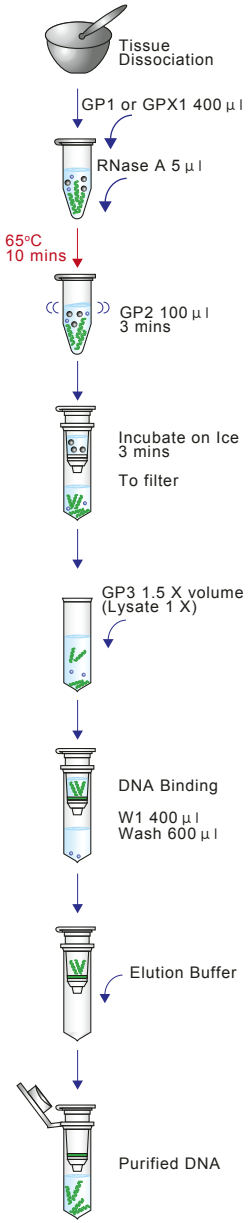
Lysis

3. Add 400 μl GP1 Buffer (or GPX1 Buffer) and 5 μl RNase A (10 mg/ml) into the sample tube and mix by vortexing. Do not mix GP1 Buffer (GPX1 Buffer) and RNase A before use.
4. Incubate at 65 ℃ for 10 minutes. During incubation, invert the tube every 5 minutes. At this time, preheat required Elution Buffer (200 μl per sample) at 65 ℃ (For DNA Elution).
5. Add 100 μl GP2 Buffer and mix by vortexing.
6. Incubate on ice for 3 minutes.
7. Place a Filter Column in a 2 ml Collection Tube.
8. Apply the mixture from previous step into the Filter Column. Centrifuge at full speed (13,000 rpm) for 3 minutes.
9. Discard the Filter Column for and carefully transfer clarified supernatant in Collection Tube to a new microcentrifuge tube (provided by user).

DNA Binding

10. Add 1.5 volumes of GP3 Buffer (isopropanol added) to 1 volume of cleared lysate and mix immediately by vortexing for 5 seconds. For example, add 750 μl GP3 Buffer to 500 μl lysate.
11. Place a GD Column in a 2 ml Collection Tube.
12. Apply 700 μl the mixture (including any precipitate) from previous step 10 into the GD Column.
13. Centrifuge at full speed (13,000 rpm) for 2 minute.
14. Discard flow-through in Collection Tube and apply remaining mixture into the GD Column.
15. Centrifuge at full speed (13,000 rpm) for 2 minute.
16. Discard flow-through in Collection Tube.





Washing

17. Add 400 µl of W1 Buffer into the GD column.
18. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
19. Discard the flow-through and place the GD Column back in the Collection Tube.
20. Add 600 µl of Wash Buffer (ethanol added) into the GD column.
21. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
22. Discard the flow-through and return the GD Column into the 2 ml Collection Tube.
23. Centrifuge again for 3 minutes at full speed to dry the column matrix.

Optional Step : Remove residue pigment
If a few pigment remain on the column matrix, perform this optional step.

a. After Wash Buffer, add 400 µl of ethanol (96-100%) into the GD column.
b. Centrifuge at 13,000 rpm for 30 seconds.
c. Discard the flow-through and place the GD Column back in the Collection Tube.
d. Centrifuge again for 3 minutes at full speed to dry the column matrix.

DNA Elution

Standard elution volume is 100 µl. If less sample volume is used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to about 200 µl.

24. Transfer dried GD Column into a clean 1.5 ml microcentrifuge tube (provided by user).
25. Add 100 µl of preheated Elution Buffer onto the center of the column matrix.
26. Stand for 3-5 minutes until Elution Buffer absorbed by the matrix.
27. Centrifuge at 13,000 rpm for 30 seconds to elute purified DNA.

ATP™ Genomic DNA Maxi Kit (Plant)

Store at room temperature (15~25℃)

Introduction

Format ： Spin columns
Sample ： 1 g plant tissue
Operation ： Centrifuge / vacuum manifold
Operation time： < 60 minutes
Yield ： Up to 500 µg
Application ： PCR、Real-Time PCR、Southern Blotting、AFLP、PADP/ AFLP

ATP™ Plant Genomic DNA Maxi Kit provides a fast and simple method to isolate total DNA (genomic DNA, mitochondrial and chloroplast) from plant tissue and cells. In the process, sample is distrusted by grinding in liquid nitrogen and lysis buffer incubation. The Lysate is treated with RNase A to degrade RNA and filtrated by filter column to remove cell debris and salt precipitations. In the presence of binding buffer with chaotropic salt, the genomic DNA in the lysate binds to glass fiber matrix in the spin column (1). The contaminants are washed with by wash buffer containing ethanol and finally, the purified genomic DNA is eluted by low salt elution buffer or water. The protocol does not require DNA phenol extraction and alcohol precipitation. The entire procedure can be completed in 60 minutes.

Quality Control

The quality of ATP™ Genomic DNA Kit (Plant) was tested on a lot-to-lot basis. The Kits were tested were isolation of genomic DNA from 500 mg young leave. The purified DNA was quantified with spectrophotometer and the yield of genomic DNA was more than 200 µg with A260/A280 ratio 1.7 to 1.9.

Kit Contents：Cat.No. / Kit Contents

AGPM25 (25 preps/kit)

GP1 Buffer ： 120 ml
GPX1 Buffer： 120 ml
GP2 Buffer ： 30 ml
GP3 Buffer*： 70 ml
W1 Buffer ： 130 ml
Wash Buffer (concentrated)**：50 ml
Elution Buffer：60 ml
RNase A (10mg/ml)：650 µl x 2
Filter Column：25 pcs
GD Column：25 pcs (green/white filter)

* add 140 ml isopropanol to GP3 Buffer prior to initial use.
** add 200 ml ethanol (96~100%) to Wash Buffer prior to initial use.

Cautions : The component contains 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).

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

Equipments and Reagents are provided by User

- ☐ 50 ml centrifuge tubes
- ☐ Centrifuge with rotor for 50 ml tubes
- ☐ 65 ℃ water-bath or dry-bath
- ☐ Grind tool
- ☐ Liquid nitrogen for sample grinding
- ☐ Ethanol (96-100 %)
- ☐ Ice

Protocol Technical Specifications

Depending on species, plant cells are extremely diverse in their cellular components, such as polysaccharides, lipids, proteins, and other metabolites.
According to this characteristic, we provide two different lysis buffers for best performances with various plant tissues.

Ⅰ. GP1 Buffer is used in the standard protocol for sample lysis. For most of common plant samples, this buffer system is sufficient to ensure purified RNA with high yields and quality.

Ⅱ. There is still an alternative buffer, Buffer GPX1, provided with the kit. The detergent in this buffer system is more effective in handling plant tissues with large quantities of polysaccharides.

Generally, these two buffer systems could achieve adequate performances. Users may try and chose one best for their demands.

Genomic DNA Maxi DNA (Plant) Protocol

Tissue Dissociation

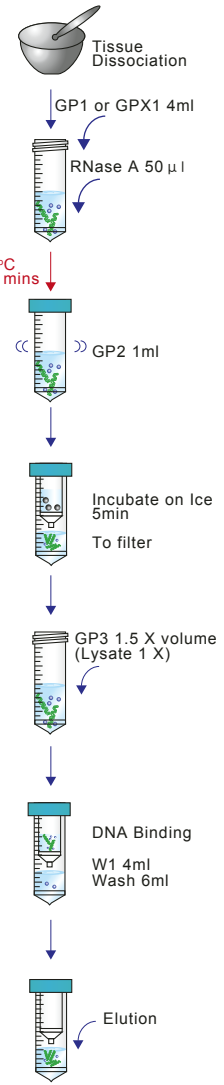
1. Cut off 0.5 g (up to 1 g) of fresh or frozen plant tissue or 50 mg (up to 100 mg) of dried sample.
2. Grind the sample under liquid nitrogen to a fine powder. Transfer it into a microcentrifuge tube (provided by user). For some plant sample, user can destruct it without liquid nitrogen.

Lysis

3. Add 4 ml GP1 Buffer (or GPX1 Buffer) and 50 μl RNase A (10mg/ml) into the sample tube and mix by vortexing. Do not mix GP1 Buffer (GPX1 Buffer) and RNase A before use.
4. Incubate at 65 ℃ for 20 minutes. During incubation, invert the tube every 5 minutes. At this time, preheat required Elution Buffer (2 ml per sample) at 65 ℃ (For DNA Elution).
5. Add 1 ml GP2 Buffer and mix by vortexing.
6. Incubate on ice for 5 minutes.
7. Place a Filter Column in a 50 ml Collection Tube.
8. Apply the mixture from previous step 5 to the Filter Column. Centrifuge at 4000 xg for 5 minutes.
9. Discard the Filter Column and carefully transfer clarified supernatant in Collection Tube to a new 50 ml centrifuge tube (provided by user).

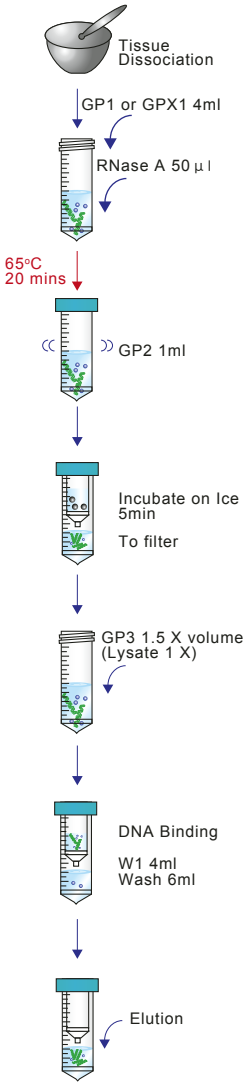
DNA Binding

10. Add 1.5 volumes of GP3 Buffer (isopropanol added) to 1 volume of cleared lysate and mix immediately by vortexing for 5 seconds. For example, add 7.5 ml GP3 Buffer to 5 ml lysate.
11. Place a GD-Maxi Column in a 50 ml Collection Tube.
12. Apply the mixture (including any precipitate) from previous step 10 into to the GD-Maxi Column.
13. Centrifuge at 4000 xg for 5 minute.
14. Discard the flow-through and apply remaining mixture into GD-Maxi Column.
15. Centrifuge at 4000 xg for 3 minute.
16. Discard the flow-through and place the GD-Maxi Column back in the Collection Tube.



ATP™ Genomic DNA Maxi Kit (Plant)

Store at room temperature (15~25℃)



Washing

17. Add 4 ml of W1 Buffer into the GD-Maxi column.
18. Centrifuge at 4000 xg for 3 minutes.
19. Discard the flow-through and place the GD-Maxi Column back in the Collection Tube.
20. Add 6 ml of Wash Buffer (ethanol added) into the GD-Maxi column.
21. Centrifuge at 4000 xg for 3 minutes.
22. Discard the flow-through and return the GD-Maxi Column in the 50 ml Collection Tube.
23. Centrifuge again for 10 minutes at 4000 xg to dry the column matrix.

Optional Step : Remove residue pigment

If a few pigment remain on the column matrix, perform this optional step.

a. After Wash Buffer, add 4 ml of ethanol (96-100%) into the GD-Maxi column.
b. Centrifuge at 4000 xg for 5 minutes.
c. Discard the flow-through and place the GD-Maxi Column back in the 50 ml Collection Tube.
d. Centrifuge again for 10 minutes at 4000 xg to dry the column matrix.

DNA Elution

Standard elution volume is 1 ml. If less sample volume is used, reduce the elution volume (200-250 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to about 2 ml.

24. Transfer dried GD-Maxi Column into a clean 50 ml centrifuge tube (provided by user).
25. Add 1 ml of preheated Elution Buffer onto the center of the column matrix.
26. Stand for 5 minutes until Elution Buffer absorbed by the matrix.
27. Centrifuge at 4000 xg for 3 minutes to elute purified DNA.

Troubleshooting

Problem	Possible Reasons / Solution
Column clogged	**Sample overloading** ・Reduce sample volume or separate into multiple tubes.
	Precipitate was formed at DNA Binding Step ・Reduce the sample material. ・Before loading the column, break up precipitate in ethanol-added lysate.
Low yield	**Incorrect DNA Elution Step** ・Ensure that Elution Buffer was added and absorbed to the center of GD Column matrix.
	Incomplete DNA Elution ・Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications.	**Residual ethanol contamination** ・Following the washing step, dry GD Column with additional centrifugation at full speed for 5minutes or incubation at 60℃ for 5 minutes.
	RNA contamination ・Perform Optional RNA Degradation Step.
	Protein contamination ・Reduce the sample amount. ・After DNA Binding Step, apply 400 µl W1 Buffer to wash GD Column and centrifuge at 13,000 rpm for 30 seconds. Proceed with Washing Step of Wash Buffer.
	Genomic DNA was degraded ・Use fresh sample, long storage may result in fragmentation of genomic DNA.

Reference

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76,615.

Note

Note

Note

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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