ATP Biotech Inc.

ATP™ Genomic DNA Maxi Kit (Blood/Culture Cell)
Catalog No. AGBM25/AGDM25

ATP™ Genomic DNA Maxi Kit (Blood/Culture Cell)

Store at room temperature (15~25℃)

Contents

Introduction

Format ： Maxi Spin column
Sample ： Up to 4 ml of fresh blood for AGBM25
Up to 10 ml of frozen blood for AGDM25
Operation ： Centrifuge / vacuum manifold
Application ： PCR、Real-Time PCR、Southern Blotting、AFLP、PADP/ AFLP

ATP™ Genomic DNA Maxi Kit (Blood/Cultured Cell) provide a fast and economical method for purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood, plasma, serum, buffy coat, other body fluids, lymphocytes, bacterial and cultured cells. In AGBM25, RBC Lysis Buffer is used to remove non-nucleated red blood cells and reduce hemoglobin contamination. In AGDM25, Proteinase K is the optimal enzyme to cell lysis for frozen blood. The method use a chaotropic salt, guanidine hydrochloride to lyse cells and degrade protein, than DNA in chaotropic salt is bond to glass-fiber matrix of column(1). After washing off the contaminants, the purified genomic DNA is eluted by low-salt elution buffer or water. The entire procedure can be completed without phenol/chloroform extraction and alcohol precipitation. Average yields of AGBM25/ AGDM25 are up to 140 μg of DNA from 4/10 ml of fresh/frozen blood. Purified DNA with approximate 20-30 kb is suitable for PCR or other enzyme reaction.

Quality Control

The quality of ATP™ Genomic DNA Maxi Kit (Blood/Culture Cell) was tested on a lot-to-lot basis. The Kits were tested by isolation of genomic DNA from 4 ml of human whole blood. The purified DNA was quantified with spectrophotometer and the yield of genomic DNA was > 100 μg with A260/A280 ratio 1.6 to 1.8.

Kit Contents：Cat.No. / Kit Contents

AGBM25 (25 preps/kit)	AGDM25 (25 preps/kit)
RBC lysis Buffer ：360 ml	Proteinase K** ：130 mg
GB Buffer ：60 ml	GB Buffer ：275 ml
W1 Buffer ：60 ml	W1 Buffer ：130 ml
Wash Buffer (concentrated)* ：25 ml	Wash Buffer (concentrated)*** ：50 ml
Elution Buffer ：60 ml	Elution Buffer ：60 ml
GD Maxi Columns ：25 pcs	GD Maxi Columns ：25 pcs

* Add 100 ml ethanol (96~100 %) to Wash Buffer prior to initial use.
** Add 13 ml ddH_2O to Proteinase K (vortex to dissolve and spin down) and store at 4℃.
*** Add 200 ml ethanol (96~100 %) to Wash Buffer prior to initial use.

Caution：GB Buffer contain guanidine hydrochloride which is 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).

Product Intended Use : Research / Clinical Application

ATP™ Genomic DNA Maxi Kit (Blood/Cultured Cell) is a research purpose device. ATP Biotech Inc. has not validated in clinical application for any organism or association, and therefore offer no specific claims for uses in diagnostics, prognostics or blood banking. This device can serve as a means for molecular assays in clinical diagnostics labratory systems after the laborartory has certified their systems according to the CLIA'88 regulation in the USA or local equivalents in other contries. Exercise all necessary care and attention when handling this product.

Equipments and Reagents are provided by User

- ☐ EDTA-NA_2-treated collection tubes (or other anticoagulant mixtures)
- ☐ 70 ℃ water-bath or dry-bath
- ☐ 50 ml centrifuge tubes
- ☐ Centrifuge With Swing-Bucket Rotor for 50 ml centrifuge tube
- ☐ PBS (phosphate-buffered saline) may be required for samples
- ☐ Ethanol (96-100%)
- ☐ RNase A (10 mg/ml, DNase-free) for Optional Step - RNA degradation

(AGBM25)Blood Maxi Protocol for Fresh Blood

For best yield, Centrifuge With Swing-Bucket Rotor is recommened for following protocol.

- ☐ Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required：70 ℃ water-bath or dry-bath、50 ml centrifuge tube、96~100 % Ethanol、RNase A (10 mg/ml)、EDTA-NA_2-treated collection tubes (or other anti-coagulant mixtures)

RBC Lysis (Use fresh blood)

1. Collected fresh blood in EDTA-NA_2-treated collection tubes (or other anti-coagulant mixtures).
2. Apply up to 4 ml of blood into a 50 ml centrifuge tube.
3. Add 3 volumes of RBC Lysis Buffer to 1 volume of the blood sample and mix by inversion. Do not vortex. (For example, add 12 ml of RBC Lysis Buffer to 4 ml blood sample.)
4. Incubate the tube for 10 min at room temperature.
5. Centrifuge at 4000 xg for 5 min and discard the supernatant.
6. Add 0.5 ml of RBC Lysis Buffer to resuspend the cell pellet.

Cell Lysis

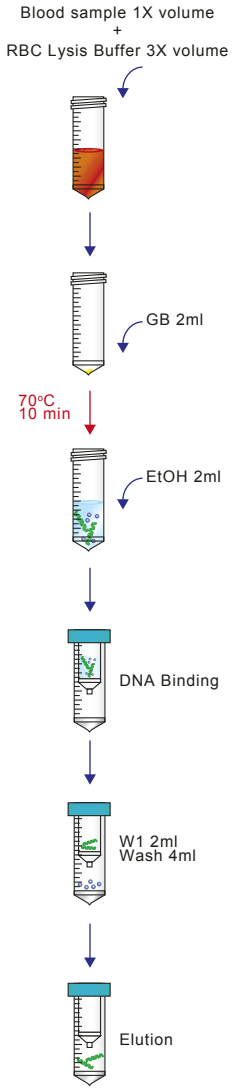
7. Add 2 ml of GB Buffer into the tube and mix by vortexing.
8. Incubate at 70 ℃ for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, preheat required Elution Buffer (2 ml per sample) at 70 ℃ (For DNA Elution).

Optional Step : RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)

- After 70 ℃ incubation, add 50 µl of RNase A (10 mg/ml, provided by user) to sample lysate and mix by vortexing.
- Incubate at room temperature for 5 minutes.

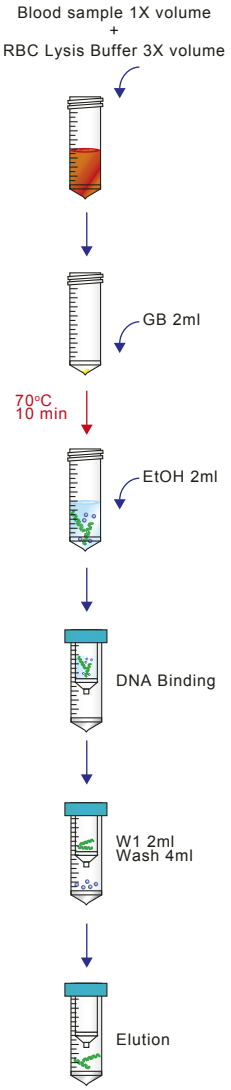
DNA Binding

9. Add 2 ml of ethanol to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
10. Place a GD-Maxi Column in a 50 ml Centrifuge Tube (provided by user).
11. Apply all the mixture (including any precipitate) from previous step into the GD-Maxi Column. Close the cap and centrifuge at 4000 xg for 5 minutes.



ATP™ Genomic Blood Maxi

AGBM25 Protocol
for Fresh Blood



Washing

12. Add 2 ml of W1 Buffer into the GD-Maxi column.
13. Centrifuge at 4,000 xg for 3 minutes.
14. Discard the flow-through and place the GD-Maxi Column back in the 50 ml Centrifuge Tube.
15. Add 4 ml of Wash Buffer (ethanol added) into the GD-Maxi Column.
16. Centrifuge at 4,000 xg for 3 minutes.
17. Discard the flow-through and return the GD-Maxi Column in the 50 ml Centrifuge Tube.
18. Centrifuge again for 10 minutes at full speed to dry the column matrix.

DNA Elution

Standard elution volume is 1 ml. If less sample volume is used, reduce the elution volume 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.

19. Transfer dried GD-Maxi Column in a clean 50 ml centrifuge tube.
20. Add 1 ml of preheated Elution Buffer onto the center of the column matrix.
21. Stand for 5 minutes until Elution Buffer has been absorbed by the matrix.
22. Centrifuge at 4000 xg for 2 minutes to elute purified DNA.

(AGDM25)Blood Maxi Protocol for Frozen Blood

For best yield, **Centrifuge With Swing-Bucket Rotor** is recommened for following protocol.

- ☐ Add 200 ml ethanol (96-100 %) to Wash Buffer prior to the initial use.
- ☐ Add 13 ml ddH_2O to Proteinase K (vortex to dissolve and spin down) and store at 4°C.
- ☐ Additionally required：70 °C water-bath or dry-bath、50 ml centrifuge tube、96~100 % Ethanol、RNase A (10 mg/ml)

Cell Lysis

1. Apply 500 µl of Proteinase K (10 mg/ml) and up to 10 ml of blood into a 50 ml centrifuge tube and mix briefly.
2. Add 10 ml of GB Buffer into the tube and mix by vortexing.
3. Incubate at 70 °C for 20 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, preheat required Elution Buffer (2.5 ml per sample) at 70 °C (For DNA Elution).

Optional Step : RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)

- After 70 °C incubation, add 50 µl of RNase A (10 mg/ml, provided by user) to sample lysate and mix by vortexing.
- Incubate at room temperature for 10 minutes.

DNA Binding

4. Add 10 ml of ethanol to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
5. Place a GD-Maxi Column in a 50 ml Centrifuge Tube.
6. Apply all the mixture (including any precipitate) from previous step into the GD-Maxi Column. Close the cap and centrifuge at 4000 xg for 5 minutes.

Washing

7. Add 4 ml of W1 Buffer into the GD-Maxi column.
8. Centrifuge at 4,000 xg for 3 minutes.
9. Discard the flow-through and place the GD-Maxi Column back into the 50 ml Centrifuge Tube.
10. Add 8 ml of Wash Buffer (ethanol added) into the GD-Maxi Column.
11. Centrifuge at 4,000 xg for 3 minutes.
12. Discard the flow-through and return the GD-Maxi Column in the 50 ml Centrifuge Tube.
13. Centrifuge again for 10 minutes at full speed to dry the column matrix.

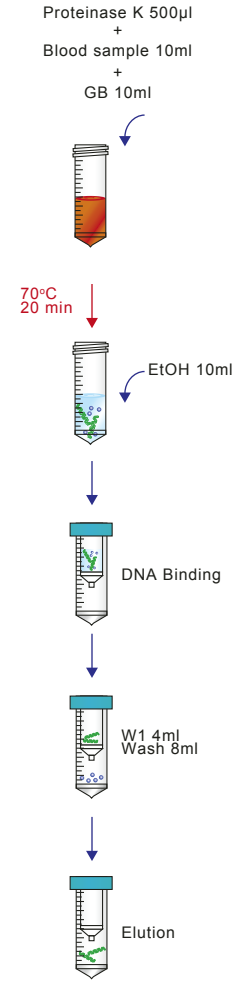
DNA Elution

Standard elution volume is 2 ml. If less sample volume is used, reduce the elution volume 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 4 ml.

14. Transfer dried GD-Maxi Column in a clean 50 ml centrifuge tube.
15. Add 2 ml of preheated Elution Buffer onto the center of the column matrix.
16. Stand for 5 minutes until Elution Buffer absorbed by the matrix.
17. Centrifuge at 4000 xg for 2 minutes to elute purified DNA.

ATP™ Genomic Blood Maxi

AGDM25 Protocol
for Frozen Blood



Note

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. ・Prior to 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-Maxi Column matrix.
	Incomplete DNA Elution ・Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications.	**Residual ethanol contamination** ・Following the wash step, dry GD-Maxi Column with additional centrifugation at full speed for 5 minutes 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 2 ml of W1 Buffer to wash GD-Maxi Column and centrifuge at 4000 xg for 3 minutes. Proceed with Washing Step.
	Genomic DNA was degraded ・Use fresh blood, long storage of samples may result in fragmentation of genomic DNA.

Reference

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

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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