ATP Biotech Inc.

ATP™ Genomic DNA Mini Kit (Blood/Culture Cell/Bacteria)
Catalog No. AGB100/AGB300

ATP™ Genomic DNA Mini Kit (Tissue)
Catalog No. AGT050/AGT300

ATP™ Genomic DNA Mini Kit (Blood/Culture Cell/Bacteria)

Store at room temperature (15~25℃)

Contents

Introduction

Format ： Spin column
Sample ： Up to 300 µl of whole blood
Up to 10^7 animal cultured cells
Up to 10^8 bacterial cultured cells
Up to 10^7 yeast
Operation ： Centrifuge / vacuum manifold
Operation time ： 20~30 minutes
Application ： PCR、Real-Time PCR、Southern blotting、AFLP、PADP/ AFLP

ATP™ Genomic DNA Mini Kit (Blood/Cultured Cell/Bacteria) 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 this procedure, RBC Lysis Buffer is used to remove non-nucleated red blood cells and reduce hemoglobin contamination. 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 in 40 minutes without phenol/chloroform extraction and alcohol precipitation. Average yield are 6 µg of DNA from 200 µl of human whole blood and up to 50 µg of DNA from 200 µl of buffy coat, 5 x 10^6 lymphocyte cells, or cultured cells. Purified DNA with approximate 20-30 kb is suitable for PCR or other enzyme reaction.

Quality Control

The quality of ATP™ Genomic DNA Kit (Blood/Cultured Cell/Bacteria) was tested on a lot-to-lot basis. The Kits were tested by isolation of genomic DNA from 200 µl of human whole blood. The purified DNA was quantified by spectrophotometer and the yield of genomic DNA was 4~6 µg with A260/A280 ratio 1.6 to 1.8.

Kit Contents：Cat.No. / Kit Contents

AGB100 (100 preps/kit)	AGB300 (300 preps/kit)
RBC lysis Buffer ：120 ml	RBC lysis Buffer ：120 ml x 3
GT Buffer ：30 ml	GT Buffer ：75 ml
GB Buffer ：30 ml	GB Buffer ：75 ml
W1 Buffer ：45 ml	W1 Buffer ：130 ml
Wash Buffer (concentrated)* ：25 ml	Wash Buffer (concentrated)** ：50 ml
Elution Buffer ：30 ml	Elution Buffer ：75 ml
GD Columns ：100 pcs (green/white filter)	GD Columns ：300 pcs (green/white filter)
Collection Tubes ：200 pcs	Collection Tubes ：600 pcs

* Add 100 ml ethanol (96~100 %) to Wash Buffer prior to initial use.
** Add 200 ml ethanol (96~100 %) to Wash Buffer prior to initial use.

Caution：GB Buffer contain 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).

Product Intended Use : Research / Clinical Application

ATP™ Genomic DNA Mini Kit (Blood/Cultured Cell/Bacteria) 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.

Recommend

- For larger volumes of whole blood or cultured cells, ATP™ Genomic DNA Blood Maxi Kits are recommended.
- For High-throughput samples processing, ATP™ 96-Well Genomic DNA Kit is recommended.
- For purification of viral RNA, the ATP™ Nucleic Acid Extraction Kit is recommended. All buffers and components of this kit are guaranteed to be RNase free.

Expected yield :

Mini	Sample	Size	DNA yield (μg)
	Blood	200 μl	4~6 μg
	Blood	1 ml	20~30 μg
	Buffy coat	200 μl	40~60 μg
	Cultured cells	5 x 10^6	15~30 μg
	Bacterial cells	2 x 10^9	20~30 μg
Maxi	**Sample**	**Size**	**DNA yield (μg)**
	Blood	10 ml	150~300 μg

Equipments and Reagents are provided by User

- ☐ Blood collection tubes with EDTA-NA_2-treated or other anticoagulant mixtures
- ☐ Microcentrifuge with rotor for 2 ml tubes
- ☐ 70 ℃ water-bath or dry-bath
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Ethanol (96-100 %)
- ☐ RNase A (10 mg/ml, DNase-free) for Optional Step - RNA degradation
- ☐ Proteinase K (10 mg/ml) for sample preparation of Frozen Blood/Whole Blood
- ☐ Lysozyme Buffer (20 mg/ml lysoyme ; 20 mM Tris-HCl ; 2 mM EDTA ; 1% Triton X-100 , pH 8.0) for prelysis of Gram-Postive bacteria. Prepare the lysozyme buffer fresh just before use.
- ☐ Sorbitol buffer (1.2 M sorbitol ; 10 mM $CaCl_2$; 0.1 M Tris-Cl , pH 7.5 ; 35 mM mercaptoethanol) for Yeast prelysis
- ☐ Lyticase or Zymolase for Yeast prelysis

Blood Mini Protocol for Fresh blood

We provide RBC Lysis Buffer to remove non-nucleated red blood cells and reduce hemoglobin contamination. But when the blood sample is less than 50 μl or sample consists of nucleated blood cells, we recommend using Cells protocol to purify genomic DNA.

- ☐ AGB100 : Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
  AGB300 : Add 200 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required : 70 ℃ water-bath or dry-bath 、Microcentrifuge tube 、96-100% Ethanol 、RNase A (10 mg/ml)

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 300 μl of blood into a 1.5 ml microcentrifuge tube. If blood sample is more than 300 μl blood (up to 1 ml), apply the blood sample into a sterile 15 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 900 μl of RBC Lysis Buffer to 300 μl blood sample.)
4. Incubate the tube for 5 min at room temperature.
5. Centrifuge at 3000 xg for 2 min and discard the supernatant.
6. Add 100 μl RBC Lysis Buffer to resuspend the cell pellet.

Cell Lysis

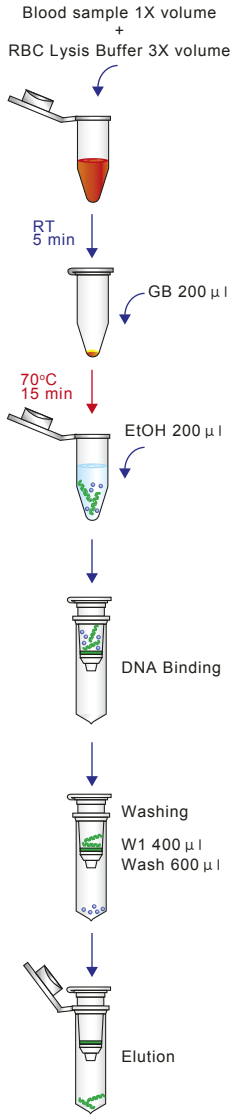
7. Add 200 μl GB Buffer into the tube and mix by vortexing.
8. Incubate at 70 ℃ for 15 minutes or until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, preheat required Elution Buffer (200 μl per sample) at 70 ℃ (For DNA Elution).

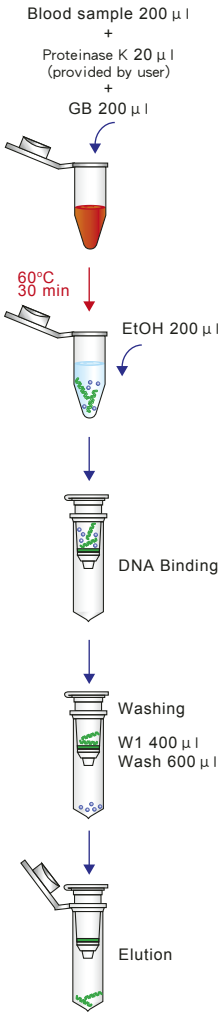
Optional Step : RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)

- After 70 ℃ incubation, add 5 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 200 μl of ethanol (96-100%) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
10. Place a GD Column in a 2 ml Collection Tube.
11. Apply all the mixture (including any precipitate) from previous step into the GD Column. Close the cap and centrifuge at full speed (13,000 rpm) for 3 minutes.





Washing

12. Add 400 µl of W1 Buffer into the GD column. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
13. Discard the flow-through and place the GD Column back in the Collection Tube.
14. Add 600 µl of Wash Buffer (ethanol added) into the GD column.
15. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
16. Discard the flow-through and return the GD Column in the 2 ml Collection Tube.
17. 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.

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

Blood Protocol For Frozen Blood or Whole Blood

☐ AGB100：Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
AGB300：Add 200 ml ethanol (96-100%) to Wash Buffer prior to the initial use.

☐ Additionally required：60 ℃ water-bath or dry-bath、Proteinase K (10 mg/ml)、Microcentrifuge tube、96~100 % Ethanol、RNase A (10 mg/ml)

Sample Preparation

1. Add 200 µl Blood + 20 µl Proteinase K (10 mg/ml, provided by user) + 200 µl GB buffer; incubate at 60 ℃ for 30 min.
2. At this time, preheat required Elution Buffer (200 µl per sample) at 60 ℃ (For DNA Elution).

Optional Step：RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)
- After 60 ℃ incubation, add 5 µl of RNase A (10 mg/ml, provided by user) to sample lysate and mix by vortexing.
- Incubate at room temperature for 5 minutes.

3. Proceed with step 9 in DNA Binding procedure of Protocol for Fresh Blood.

Culture Cells Mini Protocol

☐ AGB100：Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
AGB300：Add 200 ml ethanol (96-100%) to Wash Buffer prior to the initial use.

☐ Additionally required：70 ℃ water-bath or dry-bath、Microcentrifuge tube、96-100 % Ethanol、RNase A (10 mg/ml)

Sample Preparation

(Cultured animal cells)

If use adherent cells, trypsinize the cells before harvesting.

1. Transfer 10^6-10^7 of cells into a microcentrifuge tube (provided by user) and harvest the cells by centrifugation at 6,000 xg for 20 seconds (about 8,000 rpm for microcentrifuge).
2. Discard the supernatant and resuspend the cells with 150 µl RBC Lysis Buffer.

(Blood)

For mammalian blood (non-nucleated), the sample volume is up to 50 µl.
For nucleated erythrocytes (e.g., bird or fish), the sample volume is up to 10 µl.

1. Add 150 µl of GT Buffer into a microcentrifuge tube and apply blood sample into the tube.
2. Mix by vortexing.

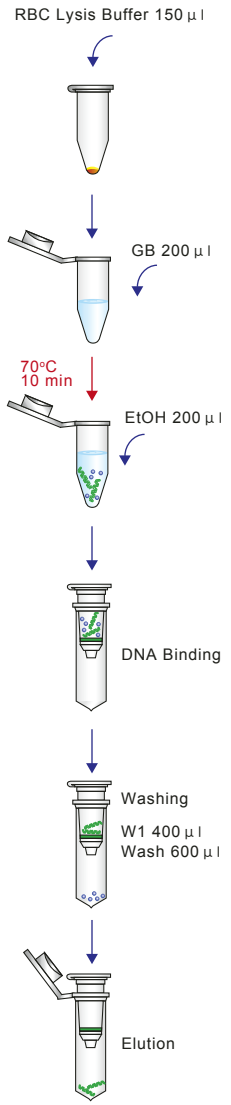
Lysis

3. Add 200 µl of GB Buffer to the sample. Mix by vortexing for 5 seconds.
4. Incubate at 70 ℃ for 10 minutes until the sample lysate is clear. During incubation, invert the tube every 3 minutes. At this time, incubate required Elution Buffer (200 µl per sample) at 70 ℃.

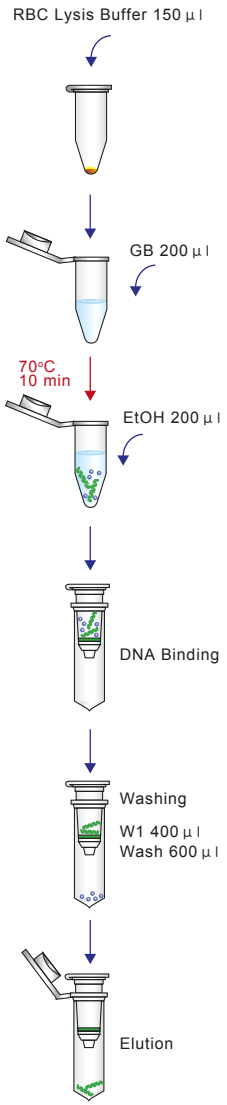
Optional Step：RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)
- After 70 ℃ incubation, add 5 µl of RNase A (10 mg/ml, provided by user) to sample lysate and mix by vortexing.
- Incubate at room temperature for 5 minutes.

DNA Binding

5. Add 200 µl of ethanol(96-100 %) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
6. Place a GD Column in a 2 ml Collection Tube.
7. Apply all the mixture (including any precipitate) from previous step into the GD Column.
8. Close the cap and centrifuge at 13,000 rpm for 3 minutes.



ATP™ Genomic DNA
(Blood/Culture Cell/Bacteria)
Culture Cells Mini Protocol



Washing

9. Add 400 µl of W1 Buffer into the GD column. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
10. Discard the flow-through and place the GD Column back in the Collection Tube.
11. Add 600 µl of Wash Buffer (ethanol added) into the GD column.
12. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
13. Discard the flow-through and return the GD Column in the 2 ml Collection Tube.
14. 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.

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

Bacterial Mini Protocol

- ☐ AGB100：Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
  AGB300：Add 200 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required：70 ℃ water-bath or dry-bath、Microcentrifuge tube、96~100 % Ethanol、RNase A (10 mg/ml)
- ☐ **For Gram-Positive bacteria**：Lysozyme Buffer (20 mg/ml lysozyme；20 mM Tris-HCl；2 mM EDTA；1 % Triton X-100，pH 8.0)，Prepare fresh lysozyme buffer just before use.

Cell harvest / Prelysis

(For Gram-Negative bacteria)

1. Transfer bacterial culture (< 10^9) into a microcentrifuge tube (provided by user). Centrifuge at full speed (13,000 rpm) for 1 min and discard the supernatant.
2. Add 200 µl of GT Buffer into the tube and resuspend the cell pellet by vortexing or pipetting. Incubate at room temperature for 5 minutes.
3. Proceed with step 3 in Cell Lysis procedure of Cultured Cells Mini Protocol.

(For Gram-Positive bacteria)

1. Transfer bacterial culture (< 10^9) into a microcentrifuge tube (provided by user). Centrifuge at full speed (13,000 rpm) for 1 min and discard the supernatant.
2. Add 200 µl of Lysozyme Buffer into the tube and resuspend the cell pellet by vortexing or pipetting. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 min.
3. Proceed with step 3 in Cell Lysis procedure of Cultured Cells Mini Protocol.

Yeast Mini Protocol

- ☐ AGB100：Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
  AGB300：Add 200 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required：
  Sorbitol buffer (1.2 M sorbitol；10 mM $CaCl_2$；0.1 M Tris-Cl，pH 7.5；35 mM β-mercaptoethanol)、Lyticase or Zymolase、70 ℃ water-bath or dry-bath、Microcentrifuge tube、96~100% Ethanol、RNase A (10 mg/ml)

Cell harvest / Prelysis

1. Harvest yeast cells (up to 5 x 10^7) by centrifugation at 5,000 xg for 10 min.
2. Discard the supernatant and resuspend the pellet in 600 µl Sorbitol Buffer.
3. Add 200 U of Lyticase or Zymolase. Incubate at 30 ℃ for 30 min.
4. Centrifuge the mixture at 2,000 xg for 10 minutes to harvest the Spheroplast.
5. Remove supernatant and add 200 µl of GT Buffer into the tube to resuspend the cell pellet by vortexing or pipetting.
6. Incubate at room temperature for 5 minutes.
7. Proceed with step 3 in Cell Lysis procedure of Cultured Cells Mini Protocol.

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 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 the standard 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.

Introduction

Format ： Spin column
Sample ： Up to 20 mg of tissue
Operation ： Centrifuge / vacuum manifold
Yield ： Up to 50 µg
Elution volume ： 50~200 µl
Application ： PCR、Real-Time PCR、Southern blotting、AFLP、PADP/ AFLP

ATP™ Genomic DNA Mini Kit (Tissue) is specially designed for purification of total DNA (including genomic, mitochondrial and viral DNA) from a variety of animal tissues or cells. Provided micropestle can efficiently homogenize tissue samples to shorten the time on the Lysis Step. The method uses proteinase K and chaotropic salt to lyse cells and degrade protein, then DNA in chaotropic salt is bound to the glass-fiber matrix of the column (1). After washing off the contaminants, the purified DNA is eluted by low salt Elution Buffer or water. The entire procedure can be completed in 60 minutes without phenol/chloroform extraction and alcohol precipitation.

Quality Control

The quality of ATP™ Genomic DNA Kit (Tissue) was tested on a lot-to-lot basis. The Kits were tested by isolation of genomic DNA from 10 mg of mouse liver. The purified DNA was quantified by 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

AGT50 (50 prep/kit)	AGT300 (300 prep/kit)
GT Buffer ：30 ml GB Buffer ：30 ml W1 Buffer ：45 ml Wash Buffer (concentrated)*：25 ml Elution Buffer：30 ml Proteinase K**：11 mg GD Columns：50 pcs Collection Tubes：100 pcs Micropestle ：50 pcs	GT Buffer ：75 ml GB Buffer ：75 ml W1 Buffer ：130 ml Wash Buffer (concentrated)***：50 ml Elution Buffer：75 ml Proteinase K****：65 mg GD Columns：300 pcs Collection Tubes：600 pcs Micropestle ：300 pcs

* Add 100 ml ethanol (96~100 %) to Wash Buffer prior to initial use.
** Add 1.1 ml ddH_2O to the tube and mix by vortexing. Store prepared Proteinase K (10 mg/ ml) at 4℃.
*** Add 200 ml ethanol (96~100 %) to Wash Buffer prior to initial use.
**** Add 6.5 ml ddH2O to the tube and mix by vortexing. Store prepared Proteinase K (10 mg/ ml) at 4 ℃.

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

Caution : GB Buffer 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).

Recommend

- For High-throughput samples processing, ATP™ 96-Well Genomic DNA Kit is recommended.
- For purification of viral RNA, the ATP™ Nucleic Acid Extraction Kit is recommended. All buffers and components of this kit are guaranteed to be RNase free.

Expected yield :

Sample	Size	DNA yield (μg)
Bacterial cells	2 x 10^9	20~30
Blood	200 μl	3~6
Brain	20mg	10~20
Buffy coat	200 μl	30~60
Cultured cells	5 x 10^6	5~30
Kidney	20mg	20~50
Liver	20mg	10~20
Lung	20mg	5~10
Mouse tail	0.5cm	10~20
Muscle	20mg	5~10

Equipments and Reagents are provided by User

- ☐ 1.5 ml microcentrifuge tubes
- ☐ Microcentrifuge with rotor for 2 ml tubes
- ☐ 60-70 ℃ water-bath or dry-bath
- ☐ Ethanol (96-100 %)
- ☐ RNase A (10 mg/ml, DNase-free; optional) for Optional Step - RNA degradation
- ☐ Xylene for sample preparation of Paraffin-embedded Tissue Protocol
- ☐ Cotton, DACRON or C.E.P. swabs for sample preparation of Buccal Swab Protocol
- ☐ PBS (phosphate-buffered saline) For Buccal Swab Protocol

Genomic DNA Mini DNA (Tissue) Protocol

- ☐ Add 1.1 ml/6.5 ml ddH_2O to a Proteinase K tube (11 mg/65 mg), vortex to dissolve. Store prepared Proteinase K at 4 ℃.
- ☐ AGT050 : Add 100 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
  AGT300 : Add 200 ml ethanol (96-100%) to Wash Buffer prior to the initial use.
- ☐ Additionally required : 60~70 ℃ water-bath or dry-bath、Microcentrifuge tube、Ethanol (96-100 %)、RNase A (10 mg/ml)

Tissue Dissociation

1. Cut up to 20 mg of animal tissue (or 0.5 cm of mouse tail) and transfer into amicrocentrifuge tube (provided by user). If used tissue has a higher number of cells (e.g., spleen or liver), reduce the starting material to 10 mg.
2. Use provided Micropestle to grind the tissue to a pulp.
3. Add 200 μl of GT Buffer into the tube and continually homogenize the sample tissue with grinding.

Lysis

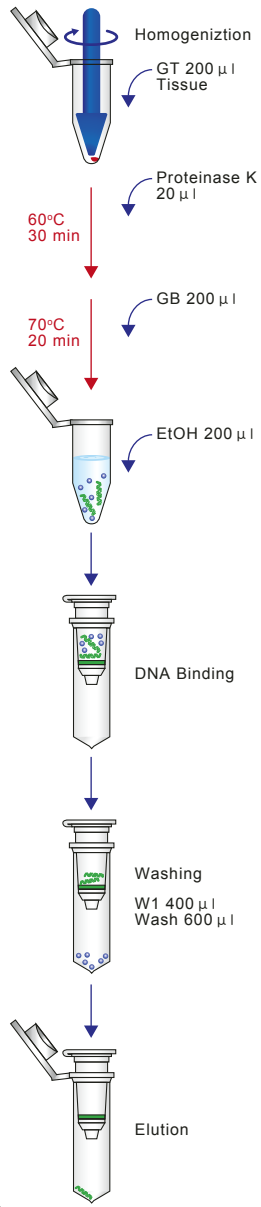
4. Add 20 μl of Proteinase K (10 mg/ml) to the sample mixture and mix by vortexing.
5. Incubate at 60 ℃ for 30 minutes. During incubation, invert the tube every 5 minutes.
6. Add 200 μl of GB Buffer and mix by vortexing for 5 seconds.
7. Incubate at 70 ℃ for 20 minutes until the sample lysate is clear. During incubation, invert the tube every 5 minutes. At this time, preheat required Elution Buffer (200 μl per sample) at 70 ℃ (For DNA Elution).
8. If there is insoluble material present following incubation, centrifuge for 2 minutes at full speed (13,000 rpm) and transfer the supernatant to a new microcentrifuge tube (provided by user).

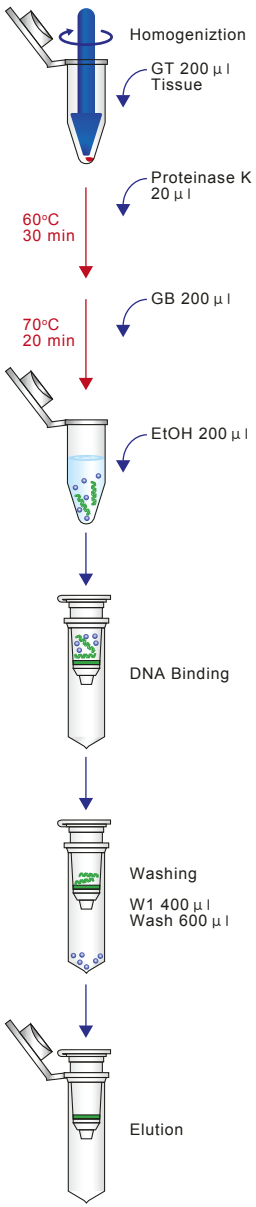
Optional Step : RNA degradation (If RNA-free genomic DNA is required, perform this optional step.)

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

DNA Binding

9. Add 200 μl of ethanol(96-100 %) to the sample lysate and mix immediately by vortexing for 10 seconds. If precipitate appears, break it up by pipetting.
10. Place a GD Column in a 2 ml Collection Tube.
11. Apply all the mixture (including any precipitate) from previous step 9 into the GD Column.
12. Centrifuge at full speed (13,000 rpm) for 2 minute.
13. Discard the Collection Tube containing the flow-through and transfer the GD Column in a new 2 ml Collection Tube.





Washing

14. Add 400 µl of W1 Buffer into the GD column.
15. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
16. Discard the flow-through and place the GD Column back in the Collection Tube.
17. Add 600 µl of Wash Buffer (ethanol added) into the GD column.
18. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
19. Discard the flow-through and return the GD Column in the 2 ml Collection Tube.
20. 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.

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

For Paraffin-embedded Tissue Protocol

☐ Additional Requirements：Xylene、37 ℃&70 ℃ water-bath or dry-bath、Ethanol(96-100 %)、Microcentrifuge tube

Sample Preparation

1. Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a microcentrifuge tube.
2. Add 1 ml xylene to each tube. Vortex vigorously and incubate at room temperature for about 10 min. Vortex occasionally during incubation step.
3. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
4. Add 1 ml ethanol(96-100 %) to wash sample pellet and mix by inverting.
5. Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
6. Repeat the ethanol(96-100 %) washing step.
7. Open the tube and Incubate at 37 ℃ for 15 minutes to evaporate the ethanol residue.
8. Proceed with step 4 in Lysis procedure of Tissue protocol.

For Buccal Swab Protocol

☐ Additional Requirements：cotton, DACRON or C.E.P. swabs、60-70 ℃ water-bath or dry-bath、PBS、Ethanol (96-100 %) 、Microcentrifuge tube.

Sample Preparation

1. Scrape the swab firmly against the inside of each cheek 6-7 times and air-dry the swab. (The person providing the sample should not eat or drink for at least 30 minutes prior to the sample collection.)
2. Separate the swab from the stick, and place the buccal swab into a 2.0 ml microcentrifuge tube.

Lysis

3. Add 500 µl of GT Buffer and 20 µl of Proteinase K (10 mg/ml) into a 1.5 ml microcentrifuge tube.
4. Incubate at 60 ℃ for 10 minutes.
5. Discard the swab and add 500 µl of GB Buffer to the lysate.
6. Mix immediately by vortexing and incubate at 60 ℃ for 10 minutes. At this time, preheat required Elution Buffer (200 µl per sample) at 60 ℃ (for DNA Elution).

DNA Binding

7. Add 500 µl of ethanol(96-100 %) to the sample lysate and mix immediately by vortexing.
8. Place a GD Column in a 2 ml Collection Tube.
9. Apply 750 µl of the mixture from previous step 7 into the GD Column.
10. Close the cap and centrifuge at full speed (13,000 rpm) for 1 minute.
11. Repeat DNA Binding Step by apply the remaining mixture to GD Column.
12. Proceed with step 14 in Washing procedure of Tissue protocol.

Troubleshooting

Problem	Possible Reasons / Solution
Column clogged	**Sample overloading** ・If using more than 20 mg of tissue, separate into multiple tubes.
	Sample tissue was not lysed completely. ・Add additional Proteinase K and extend the incubation time in Lysis step. ・After Lysis step, centrifuge for 2 minutes at full speed (13,000 rpm) to remove sample debris. Transfer the supernatant to a new microcentrifuge tube and proceed with DNA Binding Step
	Precipitate was formed at DNA Binding Step ・Reduce the sample material. ・Before loading the column, break up precipitate in ethanol-added lysate.
Low yield	**Sample tissue was not lysed completely** ・Add additional Proteinase K and extend the incubation time in Lysis Step.
	Column was clogged at DNA Binding Step ・Following the Lysis Step, remove the insoluble debris by centrifugation. ・Before loading the column, break up the precipitate in ethanol-added lysate.
	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 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 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

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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