ATP™ Genomic DNA 96-Well Kit (Tissue)

Store at room temperature (15~25℃)

Introduction

Format ：96-Well plates
Sample ：Blood, animal tissues, mouse tails, culture cells, swabs and other body fluids
Sample size： Up to 200 μl blood, 25 mg tissue or 10^6-10^7 animal cultured cells.
Operation ： Centrifuge / vacuum manifold
Operation time ： 60 minutes
Binding capacity ： Up to 30 μg per well
Application ： PCR、Real-time PCR、Southern blotting、AFLP、PADP/ AFLP

ATP™ 96-Well Genomic DNA Kit is designed for high-throughput purification of total DNA (including genomic, mitochondrial and viral DNA) from whole blood and a variety of animal tissues or cells. The method uses proteinase K and a chaotropic salt, guanidine hydrochloride to lyse cells and degrade protein, and then DNA in chaotropic salt is bonded to glass fiber matrix of plate (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 one hour without phenol/ chloroform extraction and alcohol precipitation. The kits can be used for manual filtration or with robotic handing systems and purified DNA with approximately 20-30 kb is suitable for PCR or other enzymatic reactions.

Quality Control

The quality of 96-Well Genomic DNA Kit is tested on a lot-to-lot basis. The purified DNA is checked by agarose gel analysis and quantified with spectrophotometer.

Kit Contents：Cat.No. / Kit Contents

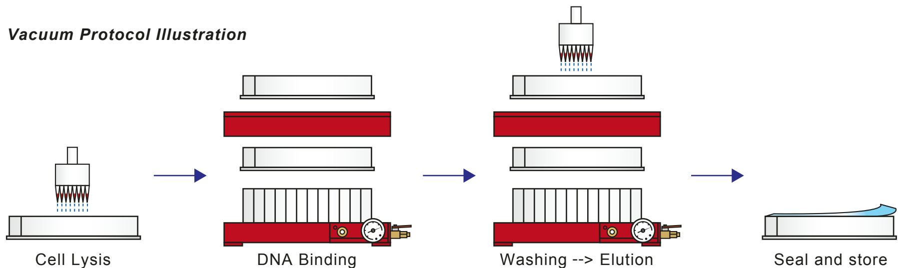
AGD9602 (2 x 96prep / kit)	AGD9604 (4 x 96prep / kit)	AGD9610 (10 x 96prep / kit)
GT Buffer ：60 ml	GT Buffer ：120 ml	GT Buffer ：240 ml
GB Buffer ：60 ml	GB Buffer ：120ml	GB Buffer ：240 ml
W1 Buffer ：130 ml	W1 Buffer ：130 ml	W1 Buffer ：130 ml x 2
Wash Buffer (concentrated)*：25 ml	Wash Buffer (concentrated)**：50 ml	Wash Buffer (concentrated)**：50ml x 2
Elution Buffer：30 ml	Elution Buffer：60 ml	Elution Buffer：60ml x 2
Proteinase K*** ：45 mg	Proteinase K*** ：45 mg x 2	Proteinase K***：45 mg x 5
96-Well DNA binding plate：2 pcs	96-Well DNA binding plate：4pcs	96-Well DNA binding plate：10pcs
0.35 ml Collection Tubes：2 pcs	0.35 ml Collection Tubes：4pcs	0.35 ml Collection Tubes：10pcs
Adhesive film：4 pcs	Adhesive film：8pcs	Adhesive film：20pcs

* Add 100 ml ethanol to Wash Buffer prior to the initial use.
** Add 200 ml ethanol to Wash Buffer prior to the initial use.
*** Add 4.5 ml ddH20 to each bottle and mix by vortexing. Store prepared Proteinase K (10 mg/ ml) at 4 ℃.

Caution：Buffers contain guanidine hydrochloride which harmful and irritant agent.
During operation, always wear a lab coat, disposable gloves, and protective goggles.

Blood Protocol

Vacuum Protocol Illustration



Cell Lysis

1. Add 200 l GB Buffer and 20 μl Proteinase K (10 mg/ ml) to each well of a 2 ml 96-Well collection plate.
2. Apply 200 μl of blood sample to each well and mix by shaking.
3. Incubate the plate at 60 ℃ for 20 minutes until the sample lysate is clear.
4. Preheat required Elution Buffer (150 μl per sample) at 60 ℃. (For DNA Elution).

DNA Binding

5. Place a 96-Well DNA Binding Plate on top of the vacuum manifold.
6. Add 200 μl of ethanol to each sample lysate in 2 ml 96-Well collection plate from precious step. Mix immediately by pipetting 5-10 times.
7. Transfer the lysate mixture to 96-Well DNA Binding Plate.
8. Apply vacuum at 10 inches Hg for 30 seconds until wells have emptied.

Washing

9. Add 300 μl of W1 Buffer to each well of the 96-Well DNA Binding Plate.
10. Apply vacuum at 10 inches Hg for 30 seconds until wells have emptied.
11. Add 600 μl of Wash Buffer (ethanol added) to each well of the 96-well DNA Binding Plate to wash again.
12. Apply vacuum at 10 inches Hg for 30 seconds until wells have emptied.
13. Apply vacuum for additional 10 min (or incubate at 60 ℃) to remove ethanol residue.

DNA Elution

Standard elution volume is 100 μl. If less sample volume is used, reduce the elution volume (50-100 μl) to increase DNA concentration.

14. Transfer the DNA Binding Plate on a clean 350 μl collection plate.
15. Add 100 μl of preheated Elution Buffer in the center of each well of DNA Binding Plate.
16. Stand for 3 minutes until Elution Buffer or water absorbed by the matrix.
17. Centrifuge for 5 min at 3,500 rpm in a centrifuge to elute purified DNA.

Additional required:

- ☐ Multi-Well Plate Vacuum Manifold
- ☐ Centrifugation system for 96-well plate
- ☐ 2 ml 96-Well collection plate

Tissue Protocol

Cell Lysis

1. Add 200 µl GT Buffer and 20 µl Proteinase K (10 mg/ ml) to each well of a 2 ml 96-Well collection plate.
2. Cut up to 20 mg of animal tissues (or 0.5 cm of mouse tail) and transfer into each well of 2 ml 96-Well collection plate (not provided).
3. Incubate the plate with shaking at 60oC for 1-2 hours to lyse the sample.

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

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

4. Add 200 µl GB Buffer to each well and mix by shaking.
5. Incubate at 70 ℃ for 20 minutes until the sample lysate is clear.
6. Preheat required Elution Buffer (50 µl per sample) at 70 ℃. (For DNA elution)
7. If there are insoluble material present following incubation, centrifuge the plate for 5 minutes at full speed and transfer the supernatants to a new 2 ml 96-Well collection plate (not provided).

DNA Binding

8. Place a 96-Well DNA Binding Plate on top of the vacuum manifold.
9. Add 200 µl of ethanol to each sample lysate in 2 ml 96-Well collection plate from precious step. Mix immediately by pipetting 5-10 times.
10. Transfer the lysate mixture to 96-Well DNA Binding Plate.
11. Apply vacuum at 10 inches Hg for 30 seconds until wells have emptied.

Washing

12. Add 300 µl of W1 Buffer to each well of the 96-Well DNA Binding Plate.
13. Apply vacuum at 10 inches Hg for 30 seconds until wells have emptied.
14. Add 600 µl of Wash Buffer (ethanol added) to each well of the 96-well DNA Binding Plate to wash again.
15. Apply vacuum at 10 inches Hg for 30 seconds until wells have emptied.
16. Apply vacuum for additional 10 min (or incubate at 60 ℃) to remove ethanol residue.

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 wash 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 Wash Step of Wash Buffer.
	Genomic DNA was degraded · Use fresh blood, long storage may result in fragmentation of genomic DNA.

Reference

(1) 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	APDA04/APDA10
ATP™ Plasmid Binding Column	50 pcs	PDC50
ATP™ Gel/PCR DNA Fragments Extraction Kit	100/300 prep	ADF100/ADF300
ATP™ 96-Well PCR Clean Up Kit	4/10 plates	APCP04/APCP10
ATP™ 96-Well Gel/PCR DAN Extraction Kit	4/10 plates	ADFP04/ADFP10
ATP™ 96-Well SEQ Dye Clean Up Kit	4/10 plates	ADCP04/ADCP10
ATP™ Fragment DNA Binding Column	50 pcs	DFC50
ATP™ Reagent Genomic DNA Kit	For 100ml blood	AGE100
ATP™ Genomic DNA Mini Kit (Blood/Culture Cell)	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)	10 prep	AGBM10
ATP™M Genomic DNA Maxi Kit (Frozen Blood)	10 prep	AGDM10
ATP™ Plant Genomic DNA Maxi Kit	10 prep	AGPM10
ATP™ 96-Well Genomic DNA Kit	4/10 plates	AGBP04/AGBP10
ATP™ Genomic DNA Binding Column	50 pcs	GDC50
ATP™ RNA Mini Kit (Blood/Culture Cell)	50prep	ARB050
ATP™ RNA Mini Kit (Tissue)	50prep	ART050
ATP™ RNA Mini Kit (Plant)	50prep	ARP050
ATP™ Viral Nucleic Acid Extraction Kit	50prep	AVR050
ATP™ RNA Maxi Kit	10 prep	ARTM10
ATP™ Plant RNA Maxi Kit	10 prep	ARPM10
ATP™ RNA Binding Column	50 pcs	RBC50