ATP Biotech Inc.

ATP™ Gel/PCR Fragment DNA Extraction Kit
Catalog No. ADF100/ADF300

ATP™ Gel/PCR Fragment DNA Extraction Kit

Store at room temperature (15~25℃)

Contents

ATP™ Gel/PCR Fragment Extraction Kit

Introduction

Format : Spin column
Sample : Up to 300 mg agarose gel slice ; Up to 100 μl PCR product or other enzymatic reaction
Operation : Centrifuge / vacuum manifold
DNA size : 50 bp ~ 10 kb
Operation time : 20 minutes for gel extraction ; 15 minutes for PCR clean up
Expected recovery : 75-85 % for gel extraction ; 80-90 % for PCR clean up
Application : DNA Sequencing ; Ligation ; PCR ; Restriction Enzyme Digestion ; DNA Labeling

ATP™ Gel/PCR Fragment DNA Extraction Kit is designed to recover or concentrate DNA fragments (50 bp-10 kb) from agarose gel, PCR or other enzymatic reaction. The method uses a chaotropic salt, guanidine thiocyanante, to dissolve the agarose gel and denature enzymes. The DNA fragments in the chaotropic salt are then bound to the uniquely designed matrix of glass-fiber in the spin column (1) in the optimized salt concentration and pH provided by our buffer. Whereas unwanted impurities, such as salts, enzymes, primers unincorporated nucleotides, dyes, and ethidium bromide flow through the column and are easily and efficiently removed from reaction mixture. After washing step, the purified DNA fragments are eluted by low-salt Elution Buffer or water. The entire procedure does not require DNA phenol extraction and alcohol precipitation, and could be completed in 20 minutes.

Quality Control

The quality of ATP™ Gel/PCR Fragment DNA Extraction Kit was tested on a lot-to-lot basis. The efficiency of DNA recovery was tested by isolation of DNA fragment of various sizes from either aqueous solution or agarose. The purified DNA was checked by agarose gel analysis.

Kit Contents : Cat.No. / Kit Contents

ADF100 (100 preps/kit)	ADF300 (300 preps/kit)
DF Buffer : 80 ml Wash Buffer (concentrated) : 25 ml* Elution Buffer : 6 ml (10mM Tris-HCl, pH 8.5 at 25 ℃) DF Columns : 100 pcs (blue/white filter) 2 ml Collection Tubes : 100 pcs	DF Buffer : 240 ml Wash Buffer (concentrated) : 50 ml** Elution Buffer : 30 ml (10mM Tris-HCl, pH 8.5 at 25 ℃) DF Columns : 300 pcs (blue/white filter) 2 ml Collection Tubes : 300 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.

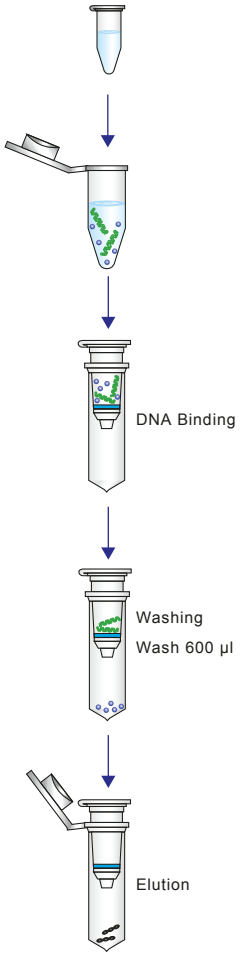
Use limitation : For research use only; not for diagnostic or medical purposes

Cautions : DF Buffer contains 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).

ATP™ Gel/PCR Kit

PCR Clean UP Protocol

PCR Product 1X volume
+
DF 5X volumes



Equipments and Reagents are provided by User

- ☐ 1.5 ml microcentrifuge tubes
- ☐ Microcentrifuge with rotor for 2 ml tubes
- ☐ 60 ℃ water-bath or dry-bath
- ☐ Ethanol (96-100%)

PCR Clean UP Protocol

Sample Preparation

1. Transfer up to 100 µl reaction products into a microcentrifuge tube (provided by user).
2. Add 5 volumes of DF Buffer to 1 volume of the sample and mix by vortexing.

DNA Binding

3. Place a DF Column in a 2 ml Collection Tube.
4. Apply the sample mixture from previous step 2 into the DF Column.
5. Centrifuge at 13,000 rpm for 30 seconds.
6. Discard the flow-through and place the DF Column back in the Collection Tube.

Washing

7. Add 600 µl of Wash Buffer (ethanol added) into the DF Column.
8. Centrifuge at 13,000 rpm for 30 seconds.
9. Discard the flow-through and place the DF Column back in the Collection Tube.
10. Centrifuge again for 3 minutes at full speed (13,000 rpm) to dry the column matrix.

DNA Elution

11. Transfer dried column in a new microcentrifuge tube (provided by user).
12. Add 15-50 µl of Elution Buffer or water onto the center of the column matrix.
13. Stand for 2 minutes until Elution Buffer or water is absorbed by the matrix.
14. Centrifuge at full speed for 2 minutes to elute purified DNA.

Gel Extraction Protocol

Gel Dissociation

1. Excise the agarose gel slice containing interested DNA fragments and remove extra agarose to minimize the size of the gel slice.
   (It is better that using TAE buffer to make the gel than TBE buffer, because TBE buffer may probably affect the downstream experiment).
2. Transfer up to 300 mg of the gel slice into a microcentrifuge tube (provided by user).
3. Add 500 µl of DF Buffer to the sample and mix by vortexing.
4. Incubate at 60 ℃ for 10-15 minutes until the gel slice completely dissolves. During incubation, invert the tube every 2-3 min.
5. Cool down the dissolved sample mixture to room temperature.

DNA Binding

6. Place a DF Column in a 2 ml Collection Tube.
7. Apply 800 µl of the sample mixture from previous step into the DF Column.
8. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
9. Discard the flow-through and place the DF Column back in the Collection Tube.
10. If the sample mixture is more than 800 µl, repeat this DNA Binding Step.

Washing

11. Add 600 µl of Wash Buffer (ethanol added) into the DF Column.
12. Centrifuge at 10,000 xg (13,000 rpm) for 30 seconds.
13. Discard the flow-through and place the DF Column back in the Collection Tube.
    Note：For TAE gels, proceed to step 14. For TBE gels, repeat steps 11-13 of Washing procedure. Boric acid is difficult to be removed and can affect downstream application, therefore double wash is recommended.
14. Centrifuge again for 3 minutes at full speed (13,000 rpm) to dry the column matrix.

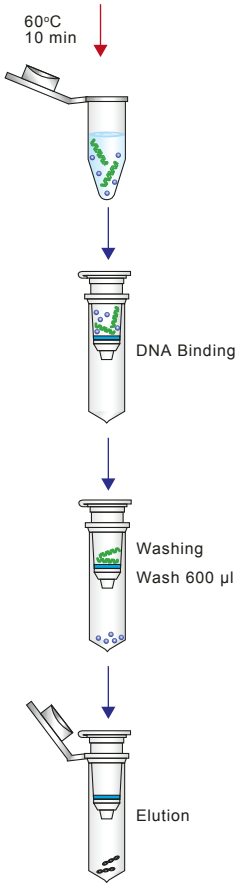
DNA Elution

15. Transfer dried column in a new microcentrifuge tube (provided by user).
16. Add 15-50 µl of Elution Buffer or water onto the center of the column matrix.
17. Stand for 2 minutes until Elution Buffer or water is absorbed by the matrix.
18. Centrifuge at full speed for 2 minutes to elute purified DNA.

ATP™ Gel/PCR Kit

Gel Extraction Protocol

Gel Slice 300 mg
+
DF 500 µl



Troubleshooting

Problem	Possible Reasons / Solution
Low yield	**Gel slice did not dissolve completely** ・Gel slice was too large. If using more than 300 mg of gel slice, separate it into multiple tubes. ・Raise temperature of incubation to 60 ℃ and extend incubation time.
	Incorrect DNA Elution Step ・Ensure that Elution Buffer was added and absorbed to the center of DF Column matrix.
	Incomplete DNA Elution ・If size of DNA fragments is larger than 10 kb, use preheated Elution Buffer (60-70 ℃) at Elution Step to improve the elution efficiency. ・If DNA is eluted by water, ensure the pH of water ranges from 7.0 to 8.5.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** ・After washing step, dry DF Column with additional centrifugation at full speed for 5 minutes or incubation at 60 ℃ for 5 minutes.
	DNA was denatured (a smaller band appeared on gel analysis) ・Incubate eluted DNA at 95 ℃ for 2 minutes, than cool down slowly to reanneal denatured 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	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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