

GenepHlow™ DNA Cleanup Midi Kit

DFI004 (4 Preparation Sample Kit)

DFI100 (100 Preparation Kit)

DFI300 (300 Preparation Kit)

Advantages

Convenient: includes pH indicator for easy determination of optimal pH and sodium acetate to adjust pH if it becomes too high

Sample: 10-40 µg of DNA in up to 200 µl PCR/Digestion solutions

Fragment Size: 100 bp-20 kb

Recovery: up to 90%

Format: DNA cleanup spin column

Operation Time: 10 minutes

Elution Volume: 30-50 µl

Kit Storage: dry at room temperature (15-25°C)

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Introduction

GenepHlow™ DNA Cleanup Midi Kits were designed to recover or concentrate DNA fragments from PCR or other enzymatic reactions. This DNA cleanup kit includes a pH indicator as an optional addition to the PB binding buffer to ensure optimal pH and facilitate DNA binding. If pH exceeds the optimal level (>7.5), the color of the solution will appear purple instead of yellow. 3M Sodium Acetate (pH5.0) which is included with the kit, can then be added to the solution to adjust pH and return the color to yellow. Chaotropic salt is used to denature enzymes while DNA fragments are bound by the glass fiber matrix of the spin column. Contaminants are removed with a Wash Buffer (containing ethanol) and the purified DNA fragments are eluted by a low salt Elution Buffer, TE or water. The pH indicator, salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture without phenol extraction or alcohol precipitation and the purified DNA is ready for use in subsequent reactions.

Quality Control

The quality of the GenepHlow™ DNA Cleanup Midi Kit is tested on a lot-to-lot basis by purifying DNA fragments of various sizes from PCR products, digestion products or other aqueous solutions. The purified DNA is analyzed by electrophoresis.

Kit Components

Component	DFI004	DFI100	DFI300
PB Buffer	4 ml	80 ml	240 ml
pH Indicator	15 µl	360 µl	1 ml
3M Sodium Acetate (pH5.0) ¹	N/A	200 µl	200 µl
Wash Buffer ² (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	6 ml	30 ml
DF Midi Columns	4	100	300
2 ml Collection Tubes	4	100	300

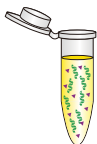
¹If the color of the mixture becomes purple instead of yellow then the pH is too high. 3M Sodium Acetate (pH5.0) can then be added to adjust pH and the color will return to yellow.

²Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

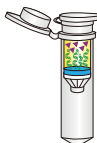


PB Buffer contains guanidine thiocyanate. During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Protocol Diagram



PB Buffer (pH \leq 7.5, yellow color when mixed with optional pH indicator) reaction of PCR or other enzymatic reaction product



DNA binding to membrane while contaminants remain suspended



Wash (removal of contaminants while DNA remains bound to membrane)



Elution of pure DNA which is ready for subsequent reactions

pH Indicator

Optimal pH



pH Too High



A pH indicator is included as an optional addition to the PB binding buffer to ensure optimal pH and facilitate DNA binding. If pH exceeds the optimal level (>7.5), the color of the solution will appear purple instead of yellow. 3M Sodium Acetate (pH5.0), which is included with the kit, can then be added to the solution to adjust pH and return the color to yellow.

GenepHlow™ DNA Cleanup Midi Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
2. It is not necessary to remove mineral oil or kerosene from the PCR sample prior to cleanup.

Additional Requirements

1.5 ml microcentrifuge tubes, absolute ethanol

DNA Cleanup Protocol Procedure

1. Sample Preparation

Optional: Add **1 ml of PB Buffer** and **4 µl of pH Indicator** to a new 1.5 ml microcentrifuge tube, then mix by shaking gently. The color will turn to yellow.

Transfer up to **200 µl of reaction product** to a 1.5 ml microcentrifuge tube. Add **5 volumes of PB Buffer (or PB Buffer premixed with pH Indicator)** to the sample then vortex.

NOTE: If the sample volume is <50 µl, adjust the sample volume to 50 µl with ddH₂O.

If the mixture has turned from yellow to purple, add 10-20 µl of 3M sodium acetate (pH5.0) and mix thoroughly. This will adjust pH and the color will return to yellow.

NOTE: If DNA fragments are >5 kb, pre-heat the required volume of Elution Buffer (50 µl/sample) to 60°C for step 4 elution.

2. DNA Binding

Place a **DF Midi Column** in a **2 ml Collection Tube**. Transfer 700 µl of the sample mixture to the **DF Midi Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the **DF Midi Column** back in the **2 ml Collection Tube**.

NOTE: If the sample mixture is >700 µl, transfer the remaining sample mixture to the DF Midi Column and repeat the DNA Binding step.

3. Wash

Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** into the **DF Midi Column** and let stand for 1 minute. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **DF Midi Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

4. Elution

Transfer the dried **DF Midi Column** to a new 1.5 ml microcentrifuge tube. Add **30-50 µl of Elution Buffer¹, TE² or water³** into the **CENTER** of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 14-16,000 x g for 2 minutes at room temperature to elute the purified DNA.

¹If DNA fragments are >5 kb, Elution Buffer should be pre-heated to 60°C. Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the DF Midi Column matrix and is completely absorbed.

²Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the DF Midi Column matrix and is completely absorbed.

³If using water for elution, ensure the water pH is ≥8.0. ddH₂O should be fresh as ambient CO₂ can quickly cause acidification. Ensure that water is added into the center of the DF Midi Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20°C to avoid degradation.

Troubleshooting



Low Yield

Incomplete Wash Buffer preparation.

Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Incorrect DNA Elution step.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the DF Midi Column matrix and is completely absorbed. If DNA fragments are larger than 5 kb, use pre-heated Elution Buffer, TE, or water (60~70°C). If using water for elution, ensure the water pH is ≥8.0. ddH₂O should be fresh as ambient CO₂ can quickly cause acidification.

GenepHlow™ DNA Cleanup Midi Kit Functional Test Data

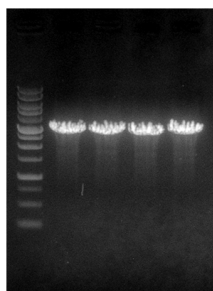


Figure 1. 40 µg of pbluescript plasmid DNA was digested by Hind III endonuclease at 37°C for 1 hour. Following digestion, the linear DNA fragment was purified using the GenepHlow™ DNA Cleanup Midi Kit. The unpurified DNA fragment (lane 1) and 3 replications of 5 µl purified linear DNA fragments (lanes 2-4) were loaded on 0.8% agarose gel.
M = Geneaid 1 Kb DNA ladder

Sample	ng/µl	260/280	260/230	Elution	Yield	Recovery
2 purified DNA	731.4	1.84	2.28	50 µl	36.6 µg	92%
3 purified DNA	724.8	1.83	2.24	50 µl	36.2 µg	91%
4 purified DNA	730.5	1.84	2.23	50 µl	36.5 µg	91%

M 1 2 3 4

Related DNA/RNA Extraction Products

Plasmid DNA Extraction		
Product	Package Size	Catalogue Number
Presto™ Mini Plasmid Kit	100/300 preps	PDH100/300
Presto™ Midi Plasmid Kit	25 preps	PIF025
Presto™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIFE25
High-Speed Plasmid Mini Kit (10-50 Kb)	100/300 preps	PDL100/300
High-Speed Plasmid Advance Kit (50-100 ml)	25 preps	PA025
Geneaid™ Midi Plasmid Kit	25 preps	PI025
Geneaid™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIE25
Presto™ Plasmid DNA Concentration Kit	250/500/1000 preps	PC0250/500/1000
Geneaid™ Maxi Plasmid Kit	10/25 preps	PM010/25
Geneaid™ Maxi Plasmid Kit (Endotoxin Free)	10/25 preps	PME10/25
Presto™ 96 Well Plasmid Kit	4/10 x 96 preps	96PDV04/10, 96PDC04/10
Post Reaction DNA Extraction		
Product	Package Size	Catalogue Number
GeneFlow™ Gel Extraction Kit	100/300 preps	DFG100/300
GeneFlow™ PCR Cleanup Kit	100/300 preps	DFC100/300
GeneFlow™ Gel/PCR Kit	100/300 preps	DFH100/300
GeneFlow™ DNA Cleanup Midi Kit	100/300 preps	DFI100/300
GeneFlow™ DNA Cleanup Maxi Kit	10/25 preps	DFM010/025
Small DNA Fragments Extraction Kit	100/300 preps	DF101/301
Presto™ Max Gel/PCR Kit (Large DNA Fragments)	100/300 preps	DFL100/300
Presto™ 96 Well PCR Cleanup Kit	4/10 x 96 preps	96DFH04/10
Presto™ 96 Well Gel Extraction Kit	4/10 x 96 preps	96FGC04/10
G-25 Gel Filtration Desalting Column	50 rxns	CG025
G-50 Gel Filtration Dye Terminator Removal Column	50 rxns	CG050
96-Well G-50 Gel Filtration Plate	4/10 x 96 rxns	CGP04/10
Genomic DNA Extraction		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	50/100/300 preps	GS050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Geneaid™ DNA Isolation Kit (Blood)	100/1,000 rxns	GEB100/01K(+)
Geneaid™ DNA Isolation Kit (Bacteria)	300/3,000 rxns	GEE300/03K(+)
Geneaid™ DNA Isolation Kit (Tissue)	150/1,500 rxns	GET150/1.5K(+)
Geneaid™ DNA Isolation Kit (Cultured Cell)	150/1,500 rxns	GEC150/1.5K(+)
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	96GBP04/10

Related DNA/RNA Extraction Products

RNA Extraction and Purification		
Product	Package Size	Catalogue Number
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Presto™ Mini RNA Bacteria Kit	50/100/300 preps	RBB050/100/300
Presto™ Mini RNA Yeast Kit	50/100/300 preps	RBV050/100/300
miRNA Isolation Kit	50/100 preps	RMI050/100
GENEzol™ Reagent	50/100/200 rxns	GZR050/100/200
GENEzol™ TriRNA Bacteria Kit	50/100 rxns	GZB050/100
GENEzol™ TriRNA Pure Kit	50/100/200 preps	GZX050/100/200
TriRNA Pure Kit	50/100/200 preps	TRP050/100/200
RNA Pure Kit	50/100 preps	PR050/100
Virus DNA/RNA Purification		
Product	Package Size	Catalogue Number
Plant Virus RNA Kit	50/100 preps	PVR050/100
Viral Nucleic Acid Extraction Kit II	50/100/300 preps	VR050/100/300
Viral Nucleic Acid Extraction Kit III	50/100/300 preps	VI050/100/300
Cloning		
Product	Package Size	Catalogue Number
Elite™ TA Cloning Kit	20 rxns	TA020
Elite™ TA Cloning Vector	20 rxns	TV020
Elite™ T4 DNA Ligase	300 U	TL100
Elite™ Competent Cells (XL1-Blue)	>5 x 10 ⁷ , 100 µl x 10, 80	CX571, CX578
Elite™ Competent Cells (XL1-Blue)	>2 x 10 ⁸ , 100 µl x 10, 80	CX281, CX288
Elite™ Competent Cells (XL1-Blue)	>5 x 10 ⁸ , 100 µl x 10, 80	CX581, CX588
Elite™ Competent Cells (DH5α)	>1 x 10 ⁸ , 100 µl x 10, 80	CD181, CD188
Elite™ Competent Cells (DH5α)	>3 x 10 ⁸ , 100 µl x 10, 80	CD381, CD388
Elite™ Competent Cells (DH5α)	>1 x 10 ⁹ , 100 µl x 10, 80	CD191, CD198
Elite™ Competent Cells BL21(DE3)	>2 x 10 ⁷ , 100 µl x 10, 80	CB271, CB278
Elite™ Competent Cells (JM109)	>5 x 10 ⁷ , 100 µl x 10, 80	CJ571, CJ578
Elite™ Competent Cells (JM109)	>1 x 10 ⁸ , 100 µl x 10, 80	CJ181, CJ188
DNA Ladders and Markers		
Product	Package Size	Catalogue Number
100 bp DNA Ladder	50 µg, 500 µl	DL007
1 Kb DNA Ladder	50 µg, 500 µl	DL006
Loading Dye (6X)	10/100 ml	LD010/100

For additional product information please visit www.geneaid.com. Thank you!

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