Geneaid

Instruction Manual
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For Research Use Only

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 aqeous solutions. The purified DNA is analyzed by electrophoresis.

Kit Components

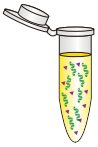
Component	DFI004	DFI100	DFI300
PB Buffer	4 ml	80 ml	240 ml
pH Indicator	15 µl	360 µl	1 ml
3M Sodium Acetate (pH5.0)[1]	N/A	200 µl	200 µl
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	6 ml	30 ml
DF Midi Columns	4	100	300
2 ml Collection Tubes	4	100	300

[1]If 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.

[2]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.

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

Quick Protocol Diagram



PB Buffer (pH≤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 ddH_2O.

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 60ºC 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 Buffer**[1], TE[2] or water[3] 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.

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

[2]Using 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.

[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 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 -20ºC 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 (60~70ºC). If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

GenepHlow™ DNA Cleanup Midi Kit Functional Test Data

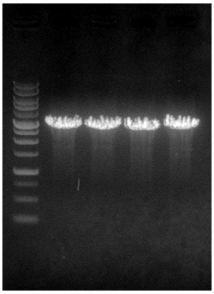


Figure 1. 40 µg of pbluescript plasmid DNA was digested by Hind III endonuclease at 37ºC for 1 hour. Following digestion, the linear DNA fragment was purified using the GenepHlow™ DNA Cleanup Midi Kit. The unpurified DNA fragment (lane 1) and 3 replications of 5 µl purified linear DNA fragments (lanes 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%

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