Geneaid

Instruction Manual
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For Research Use Only

GenepHlow™ DNA Cleanup Maxi Kit

DFM002 (2 Preparation Sample Kit)
DFM010 (10 Preparation Kit)
DFM025 (25 Preparation Kit)

Advantages

Efficient: purify DNA fragments from large sample volumes within 30 minutes
Sample: up to 5 g of agarose gel, up to 2 ml of restriction enzyme digestion product or PCR product
Fragment Size: 100 bp-10 kb
Recovery: up to 80% for enzymatic reaction cleanup, up to 70% for gel extraction
Format: maxi spin column
Convenient: includes pH indicator for easy determination of optimal pH and sodium acetate to adjust pH if it becomes too high following Gel/PCR Buffer reaction
Binding Capacity: 200 µg
Time: 24 minutes for enzymatic reaction cleanup and 36 minutes for gel extraction
Elution Volume: 500-1,000 µl (50 µl dead volume)
Kit Storage: dry at room temperature (15-25°C) for up to 1 year

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Introduction

GenepHlow™ DNA Cleanup Maxi Kits were designed to recover or concentrate DNA fragments from from large volume agarose gel, restriction enzyme digestion or PCR reaction products. Gel/PCR Buffer (yellow color indicating optimal pH≤7.5) is premixed with a pH indicator to ensure optimal pH, facilitate DNA binding and allow for easy observation of undissolved agarose gel. 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 dissolve agarose gel and 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 Maxi Kits is tested on a lot-to-lot basis by purifying DNA fragments from either agarose gel, restriction enzyme digestion products, PCR products or other aqueous solutions and analyzed by electrophoresis.

Kit Components

Component	DFM002	DFM010	DFM025
Gel/PCR Buffer	25 ml	125 ml	275 ml
3M Sodium Acetate (pH5.0)[1]	N/A	2 ml	2 ml
W1 Buffer	15 ml	75 ml	175 ml
Wash Buffer[2] (Add Ethanol)	5 ml (20 ml)	25 ml (100 ml)	50 ml + 25 ml (200 ml) (100 ml)
Elution Buffer	2 ml	12 ml	30 ml
DM Columns	2	10	25

[1]If the color of the mixture becomes purple instead of yellow once the gel slice is dissolved completely or following enzymatic product reaction, 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.

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

Quick Protocol Diagram

Gel/PCR Buffer (pH≤7.5, yellow color, premixed with pH indicator) reaction of gel slice or 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 premixed with the Gel/PCR Buffer to ensure optimal pH, facilitate DNA binding and allow for easy observation of undissolved agarose gel. 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 Maxi Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.
During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

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. Record the weight of an empty 15 ml centrifuge tube for the Gel Dissociation step. Once the gel has been transferred to the tube, record the weight again. Subtract the empty tube weight from the total weight to determine the actual gel weight.
3. It is not necessary to remove mineral oil or kerosene from the PCR sample prior to cleanup.

Additional Requirements
15 ml centrifuge tube, 50 ml centrifuge tube, absolute ethanol

Gel Extraction Protocol Procedure

1. Gel Dissociation

Cut the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. Transfer **up to 5 g of the gel slice** to a 15 ml centrifuge tube. Add **8 ml of Gel/PCR Buffer** to the sample then mix by vortex. Incubate at 55-60ºC for 10-15 minutes or until the gel slice is completely dissolved. During incubation, invert the tube every 2-3 minutes. If the color of the mixture has turned from yellow to purple, add 200 µl of 3M Sodium Acetate (pH5.0) and mix thoroughly. This will adjust pH and the color will return to yellow. Cool the dissolved sample mixture to room temperature. NOTE: Use ≤2% agarose gel to ensure optimal dissolution efficiency and DNA yield.

2. DNA Binding

Place a **DM Column** in a 50 ml centrifuge tube. Transfer **6 ml of the sample mixture** to the **DM Column** (DO NOT load more than 6 ml of the sample mixture to the **DM Column** to prevent spilling during centrifugation). Centrifuge at 5-6,000 x g for 2 minutes. Discard the flow-through then place the **DM Column** back in the 50 ml centrifuge tube. Transfer the remaining sample mixture to the same **DM Column** and centrifuge at 5-6,000 x g for 2 minutes. Discard the flow-through then place the **DM Column** back in the 50 ml centrifuge tube.

3. Wash

Add **6 ml of W1 Buffer** into the **DM Column**. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Add **6 ml of Wash Buffer (make sure absolute ethanol was added)** into the **CENTER** of the **DM Column** and let stand for 3 minutes at room temperature. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Add **6 ml of Wash Buffer (make sure absolute ethanol was added)** into the **CENTER** of the **DM Column**. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Centrifuge at 5-6,000 x g for 5 minutes to dry the column matrix.

4. Elution

Transfer the dried **DM Column** to a new 50 ml centrifuge tube. Add **0.5-1 ml of Elution Buffer**[1], TE[2] or water[3] into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 5-6,000 x g for 5 minutes at room temperature to elute the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the DM 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 DM 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 DM Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Enzymatic Reaction Cleanup Protocol Procedure

1. Sample Preparation

Transfer up to **2 ml of reaction product** to a 15 ml centrifuge tube. Add **5 volumes of Gel/PCR Buffer** to the sample then vortex. If the mixture has turned from yellow to purple, add 200 µl of 3M sodium acetate (pH5.0) and mix thoroughly. This will adjust pH and the color will return to yellow.

2. DNA Binding

Place a **DM Column** in a 50 ml centrifuge tube. Transfer **6 ml of the sample mixture** to the **DM Column** (DO NOT load more than 6 ml of the sample mixture to the **DM Column** to prevent spilling during centrifugation). Centrifuge at 5-6,000 x g for 2 minutes. Discard the flow-through then place the **DM Column** back in the 50 ml centrifuge tube. Transfer the remaining sample mixture to the same **DM Column** and centrifuge at 5-6,000 x g for 2 minutes. Discard the flow-through then place the **DM Column** back in the 50 ml centrifuge tube.

3. Wash

Add **6 ml of Wash Buffer (make sure absolute ethanol was added)** into the **CENTER** of the **DM Column** and let stand for 3 minutes at room temperature. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Add **6 ml of Wash Buffer (make sure absolute ethanol was added)** into the **CENTER** of the **DM Column**. Centrifuge at 5-6,000 x g for 2 minutes then discard the flow-through. Place the **DM Column** back in the 50 ml centrifuge tube. Centrifuge at 5-6,000 x g for 5 minutes to dry the column matrix.

4. Elution

Transfer the dried **DM Column** to a new 50 ml centrifuge tube. Add **0.5-1 ml of Elution Buffer**[1], TE[2] or water[3] into the **CENTER** of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 5-6,000 x g for 5 minutes at room temperature to elute the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the DM 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 DM 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 DM Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

Low Yield

Agarose gel did not dissolve completely.

Ensure the agarose gel was melted/dissolved completely between 55-60ºC for 10-15 minutes, or until no gel is visible. If undissolved agarose remains in the sample, the DM Column could clog and some DNA will be unrecoverable. DNA can be denatured if the incubation temperature exceeds 60ºC. If using more than 5 g of agarose gel, separate it into multiple 15 ml centrifuge tubes. Use ≤2% agarose gel to ensure optimal dissolution efficiency and DNA 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

Incorrect DNA elution step.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the DM 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.

Eluted DNA Does Not Perform Well In Downstream Applications

DNA was denatured (a smaller band appeared on gel analysis).

DNA can be denatured if the incubation temperature exceeds 60ºC. Incubate the eluted DNA at 95ºC for 2 minutes then cool down slowly to re-anneal the denatured DNA.

Primer dimer contamination in the final PCR elution product.

Gel purification should be performed if primer dimers are visible in the agarose gel following PCR reactions. Simply cut the PCR product from the gel and avoid the primer dimer. Using an additional 80% ethanol wash will reduce primer dimer contamination when performing PCR cleanup.

GenepHlow™ DNA Cleanup Maxi Kit Functional Test Data

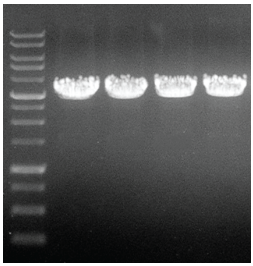

Figure 1. 200 µg of pbluescript plasmid DNA was digested by *Hind* III endonuclease at 37ºC for 2 hours. Following digestion, the linear DNA fragment was purified using the GenepHlow™ DNA Cleanup Maxi Kit. The unpurified DNA fragment (lane 1) and 3 replications of 5 µl purified linear DNA fragments (lanes 2-4) were loaded on 0.8% agarose gel. M = Geneaid 1 Kb DNA ladder

Sample	ng/µl	260/280	260/230	Elution	Yield (µg)	Recovery
2 – Purified DNA	155.4	1.87	2.17	1000 µl	155.4	77.7%
3 – Purified DNA	157.8	1.86	2.22	1000 µl	157.8	78.9%
4 – Purified DNA	152.6	1.87	2.24	1000 µl	152.6	76.3%

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