Geneaid

Instruction Manual
Ver. 02.08.18
For Research Use Only

GenepHlow™ Gel Extraction Kit

DFG004 (4 Preparation Sample Kit)
DFG100 (100 Preparation Kit)
DFG300 (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 following gel dissociation
Sample: up to 300 mg of agarose gel
Fragment Size: 70 bp-20 kb
Recovery: up to 90%
Format: gel extraction spin column
Operation Time: 20 minutes
Elution Volume: 20-50 µl
Kit Storage: dry at room temperature (15-25°C)

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Introduction

GenepHlow™ Gel Extraction Kits were designed to recover or concentrate DNA fragments from agarose gel. QG 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™ Gel Extraction Kit is tested on a lot-to-lot basis by purifying DNA fragments of various sizes from agarose gel. The purified DNA is analyzed by electrophoresis.

Kit Components

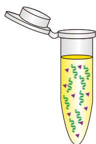
Component	DFG004	DFG100	DFG300
QG Buffer	3 ml	80 ml	240 ml
3M Sodium Acetate (pH5.0)[1]	N/A	200 µl	200 µl
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[2] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml + 25 ml (200 ml) (100 ml)
Elution Buffer	1 ml	6 ml	30 ml
DFH Columns	4	100	300
2 ml Collection Tubes	4	100	300

[1]If the color of the mixture becomes purple instead of yellow once the gel slice is dissolved completely 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.

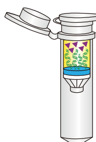
[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

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

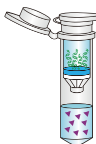
Quick Protocol Diagram



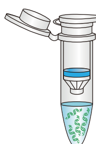
QG Buffer (pH≤7.5, yellow color, premixed with pH indicator) reaction of gel slice



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 QG 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.

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GenepHlow™ Gel Extraction Kit Protocol 1 of 2

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. Record the weight of an empty 1.5 ml microcentrifuge 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. Perform gel purification when primer dimers are highly visible or add an additional 80% ethanol wash to avoid primer dimer contamination.

Additional Requirements

1.5 ml microcentrifuge tubes, 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 300 mg of the gel slice** to a 1.5 ml microcentrifuge tube. Add **500 µl of QG 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 10 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: If using less than 300 mg of gel slice, QG Buffer does not need to be scaled. If using more than 300 mg of gel slice, separate it into multiple 1.5 ml microcentrifuge tubes. Use ≤2% agarose gel to ensure optimal dissolution efficiency and DNA yield.

2. DNA Binding

Place a **DFH Column** in a 2 ml Collection Tube. Transfer **800 µl of the sample mixture** to the DFH Column. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the **DFH Column** back in the 2 ml Collection Tube.

NOTE: If the sample mixture is more than 800 µl, repeat the DNA Binding Step.

3. Wash

Add **400 µl of W1 Buffer** into the **DFH Column**. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **DFH Column** back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure absolute ethanol was added)** into the **DFH Column** and let stand for 1 minute. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **DFH 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 **DFH Column** to a new 1.5 ml microcentrifuge tube. Add **20-50 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]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the DFH 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 DFH 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 DFH Column matrix and is completely absorbed. DNA eluted in water should be stored at -20ºC to avoid degradation.

GenepHlow™ Gel Extraction Kit Protocol 2 of 2

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. Record the weight of an empty 1.5 ml microcentrifuge 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. Perform gel purification when primer dimers are highly visible or add an additional 80% ethanol wash to avoid primer dimer contamination.

Additional Requirements

1.5 ml microcentrifuge tubes, absolute ethanol

Gel Extraction Protocol Procedure for Sequencing

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 300 mg of the gel slice** to a 1.5 ml microcentrifuge tube. Add **500 µl of QG 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 10 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: If using less than 300 mg of gel slice, QG Buffer does not need to be scaled. If using more than 300 mg of gel slice, separate it into multiple 1.5 ml microcentrifuge tubes. Use ≤2% agarose gel to ensure optimal dissolution efficiency and DNA yield.

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2. DNA Binding

Place a **DFH Column** in a 2 ml Collection Tube. Transfer **800 μl of the sample mixture** to the **DFH Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the **DFH Column** back in the 2 ml Collection Tube.

NOTE: If the sample mixture is more than 800 μl, repeat the DNA binding step.

3. Wash

Add **600 μl of Wash Buffer (make sure absolute ethanol was added)** into the **DFH Column** and let stand for 1 minute. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **DFH Column** back in the 2 ml Collection Tube. Add **600 μl of Wash Buffer (make sure absolute ethanol was added)** into the **DFH Column** and let stand for 1 minute. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **DFH 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 **DFH Column** to a new 1.5 ml microcentrifuge tube. Add **20-50 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]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the DFH 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 DFH 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 DFH 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 DFH Column could clog and some DNA will be unrecoverable. DNA can be denatured if the incubation temperature exceeds 60ºC. If using more than 300 mg of agarose gel, separate it into multiple 1.5 ml microcentrifuge 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 to avoid ethanol evaporation.

Incorrect DNA elution step.

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

Ensure the agarose gel was melted/dissolved completely between 55-60ºC for 10-15 minutes, or until no gel is visible. DNA can be denatured if the incubation temperature exceeds 60ºC. Incubate the eluted DNA at 95ºC for 2 minutes then cool down slowly to reanneal 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™ Gel Extraction Kit Functional Test Data

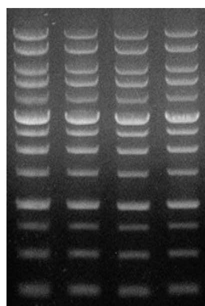

Figure 1. Gel slice DNA fragments ranging from 250 bp-10 kb were extracted using the GenepHlow™ Gel Extraction Kit (lane 1, 2, 3). The purified DNA from a 50 µl eluate was analyzed by electrophoresis on a 1% agarose gel.

M = Geneaid 1 Kb DNA Ladder (control, total DNA = 1100 ng)

Test	DNA Conc.	260/280	Yield	Recovery
1	18.2 ng/µl	1.84	910 ng	82%
2	18.7 ng/µl	1.82	935 ng	85%
3	19.2 ng/µl	1.82	960 ng	87.3%

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CERTIFICATE NO. QAIC/TW/50077