GenepHlow™ Gel/PCR Kit Quick Protocol

For research use only

Catalogue Number

DFH004, DFH100, DFH300

Instruction Manual Download

When using this product for the first time, or if you are unfammiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

Gel Extraction Protocol (See page 2 for Sequencing Protocol)

1. Gel Dissociation

Excise 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 Gel/PCR Buffer** and mix by vortex. Incubate at 55-60ºC for 10-15 minutes to ensure the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 minutes. If the color of the mixture has turned to purple, add 10 µl of 3M Sodium Acetate (pH5.0) and mix thoroughly. Cool the dissolved sample to room temperature.

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 and place the **DFH Column** back in the **2 ml Collection Tube**. 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 ethanol was added) into the **DFH Column**. Let stand for 1 minute at room temperature. 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. DNA Elution

Transfer the dried **DFH Column** to a new 1.5 ml microcentrifuge tube. Add **20-50 µl of (60-70ºC) pre-heated Elution Buffer** or TE into the center of the column matrix. Let stand for at least 2 minutes to ensure the **Elution Buffer** is completely absorbed. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.

PCR Cleanup Protocol

1. Sample Preparation

Transfer up to **100 µl of reaction product** to a 1.5 microcentrifuge tube. If the sample is less than 50 µl, adjust the volume to 50 µl with ddH_2O. Add 5 volumes of **Gel/PCR Buffer** to 1 volume of the sample and mix by vortex. If the color of the mixture has turned to purple, add 10 µl of 3M Sodium Acetate (pH5.0) and mix thoroughly.

2. DNA Binding

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

3. Wash

Add **600 µl of Wash Buffer** (make sure ethanol was added) into the center of the **DFH Column**. Let stand for 1 minute at room temperature. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the **DFH Column** back in the **2 ml Collection Tube**. Centrifuge for 3 minutes at 14-16,000 x g to dry the column.

4. DNA Elution

Transfer the dried **DFH Column** to a new 1.5 ml microcentrifuge tube. Add **20-50 µl of (60-70ºC) pre-heated Elution Buffer** or TE into the center of the column matrix. Let stand for at least 2 minutes to ensure the **Elution Buffer** is completely absorbed. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.

Gel Extraction For Sequencing Protocol

1. Gel Dissociation

Excise 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 Gel/PCR Buffer** and mix by vortex. Incubate at 55-60ºC for 10-15 minutes to ensure the gel slice has been completely dissolved. During incubation, invert the tube every 2-3 minutes. If the color of the mixture has turned to purple, add 10 µl of 3M Sodium Acetate (pH5.0) and mix thoroughly. Cool the dissolved sample mixture to room temperature.

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 and place the **DFH Column** back in the **2 ml Collection Tube**. If the sample mixture is more than 800 µl, repeat the DNA Binding step.

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Add **600 µl of Wash Buffer** (make sure 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 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. DNA Elution

Transfer the dried **DFH Column** to a new 1.5 ml microcentrifuge tube. Add **20-50 µl of (60-70ºC) pre-heated Elution Buffer** or TE into the center of the column matrix. Let stand for at least 2 minutes to ensure the **Elution Buffer** is completely absorbed. Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.

Components

Component	DFH004	DFH100	DFH300
Gel/PCR Buffer	3 ml	80 ml	240 ml
3M Sodium Acetate (pH5.0)	N/A	200 µl	200 µl
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer (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

Storage

Dry at room temperature (15-25ºC)

NOTE

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.