Gel/PCR DNA Fragments Extraction Kit

For research use only

Sample: up to 300 mg of agarose gel, up to 100 µl of PCR products

Fragment Size: 70 bp – 20 kb

Recovery: up to 95%

Format: spin column

Operation Time: 20 minutes (gel extraction), 10 minutes (PCR cleanup)

Elution Volume: 20-50 µl

Storage: dry at room temperature (15-25ºC)

Introduction

The Gel/PCR DNA Fragments Extraction Kit was designed to recover or concentrate DNA fragments (70 bp-20 kb) from agarose gel, PCR, or other enzymatic reactions in one convenient product. Chaotropic salt is used to dissolve agarose gel and denature enzymes. DNA fragments in chaotropic salt are bound by the glass fiber matrix of the spin column (1). Contaminants are removed with a Wash Buffer (containing ethanol) and the purified DNA fragments are eluted by a low salt Elution Buffer or TE. Salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture, without phenol extraction or alcohol precipitation. Typically, recoveries are up to 90% for Gel Extraction and up to 95% for PCR Clean Up. The eluted DNA is ready for use in PCR, Fluorescent or Radioactive Sequencing, Restriction Enzyme Digestion, DNA Labeling and Ligation.

Quality Control

The quality of the Gel/PCR DNA Fragments Extraction Kit is tested on a lot-to-lot basis by isolating DNA fragments of various sizes from either aqueous solutions or agarose gel. The purified DNA is analyzed by electrophoresis.

Kit Contents

Component	IB47010	IB47020	IB47030
DF Buffer	3 ml	80 ml	240 ml
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
DF Columns	4	100	300
2 ml Collection Tubes	4	100	300

*Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use.

Caution

DF Buffer contains guanidine thiocyanate. During operation, always wear a lab coat, disposable gloves, and protective goggles.

PCR Clean Up Protocol

IMPORTANT BEFORE USE!

• Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use

• Additional Requirements: absolute ethanol, microcentrifuge tubes

Sample Prep.	• Transfer **up to 100 µl of reaction product** to a 1.5 microcentrifuge tube. • Add **5 volumes of DF Buffer** to 1 volume of the sample and mix by vortex.
Step 1 DNA Binding	• Place a **DF Column** in a **2 ml Collection Tube**. • Transfer **the sample mixture** to the **DF Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the **DF Column** back in the **2 ml Collection Tube**.
Step 2 Wash	• Add **600 µl of Wash** Buffer **(make sure ethanol was added)** into the CENTER of the **DF 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 **DF Column** back in the **2 ml Collection Tube**. • Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 3 DNA Elution	• Transfer the dried **DF Column** to a new 1.5 ml microcentrifuge tube. • Add **20-50 µl of 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. NOTE: Using pre-heated Elution Buffer (60ºC) is recommended for eluting DNA fragments >5kb.

Gel Extraction Protocol

IMPORTANT BEFORE USE!

• Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use.
• Additional Requirements: absolute ethanol, microcentrifuge tubes

Gel Dissociation	• Excise the agarose gel slice containing relevant DNA fragments and Remove any extra agarose. NOTE: Using TAE buffer for gel formation is recommended for optimal DNA recovery. • Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube then Add **500 µl of DF Buffer** and vortex. • Incubate at 55-60ºC for 10-15 minutes to ensure the gel slice has been completely dissolved. NOTE: During incubation, invert the tube every 2-3 minutes. • Cool the dissolved sample mixture to room temperature.
Step 1 DNA Binding	• Place the **DF Column** in a **2 ml Collection Tube** then transfer **800 µl of the sample mixture** to the **DF Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **DF Column** back in the **2 ml Collection Tube**. NOTE: If the sample mixture is more than 800 µl, repeat the DNA Binding step.
Step 2 Wash	• Add **400 µl of W1 Buffer** into the **DF Column** then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **DF Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **DF Column**. • Let stand for 1 minute at room temperature then centrifuge at 14-16,000 x g for 30 seconds. • discard the flow-through then place the **DF Column** back in the **2 ml Collection Tube**. • Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.
Step 3 DNA Elution	• Transfer the dried **DF Column** to a new 1.5 ml microcentrifuge tube. • Add **20-50 µl of 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. NOTE: Using pre-heated Elution Buffer (60ºC) is recommended for eluting DNA fragments >5kb.

Gel Extraction For Sequencing Protocol

IMPORTANT BEFORE USE!

• Add absolute ethanol (see the bottle label for volume) to Wash Buffer prior to initial use
• Additional Requirements: absolute ethanol, microcentrifuge tubes
• Please use this protocol for sequencing as Guanidinium Chloride, a component of W1 Buffer, may interfere with sequencing reactions.

Gel Dissociation	• Excise the agarose gel slice containing relevant DNA fragments and remove any extra agarose. NOTE: Using TAE buffer for gel formation is recommended for optimal DNA recovery. • Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube then add **500 µl of DF Buffer** and vortex. • Incubate at 55-60ºC for 10-15 minutes to ensure the gel slice has been completely dissolved. NOTE: During incubation, invert the tube every 2-3 minutes. • Cool the dissolved sample mixture to room temperature.
Step 1 DNA Binding	• Place the **DF Column** in a **2 ml Collection Tube** then transfer **800 µl of the sample mixture** to the **DF Column**. • Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. • Place the **DF Column** back in the **2 ml Collection Tube**. NOTE: If the sample mixture is more than 800 µl, repeat the DNA Binding Step.
Step 2 Wash	• Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **DF 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 Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **DF 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 Column** back in the **2 ml Collection Tube**. • Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.
Step 3 DNA Elution	• Transfer the dried **DF Column** to a new 1.5 ml microcentrifuge tube. • Add **20-50 µl of 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. NOTE: Using pre-heated Elution Buffer (60ºC) is recommended for eluting DNA fragments >5kb.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	**Gel slice did not dissolve completely** • The Gel slice was too big. If using more than 300 mg of gel slice, separate it into multiple tubes. • Raise the incubation temperature to 60ºC and extend the incubation time.
	Incorrect DNA Elution Step • Ensure that the Elution Buffer is completely absorbed after being added to the center of the DF Column.
	Incomplete DNA Elution • If DNA fragments are larger than 5 kb, use pre-heated Elution Buffer (60-70ºC) to improve the elution efficiency.
DNA doesn't perform well in downstream applications	**Residual ethanol contamination following Wash Step** • Dry the DF Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60ºC for 5 minutes.
	DNA was denatured (a smaller band appeared on gel analysis) • Incubate the eluted DNA at 95ºC for 2 minutes, and then cool down slowly to re-anneal the denatured DNA.
Low A260/A230	• In the wash step, repeat the 600 µl of Wash Buffer addition and let stand for 1 minute.