

For Research Use Only



INSTRUCTION

MANUAL

Gel/PCR DNA Fragments Extraction Kit

IB47010 (4 Preparation Kit)
IB47020 (100 Preparation Kit)
IB47030 (300 Preparation Kit)

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 (15-25°C)

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 (60°C) 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 55-60°C 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 (60°C) 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 55-60°C 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 (60°C) is recommended for eluting DNA fragments >5kb.

Troubleshooting

Component	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 60°C 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 (60-70°C) 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 60°C for 5 minutes. DNA was denatured (a smaller band appeared on gel analysis) • Incubate the eluted DNA at 95°C 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.



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