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

Recovery : up to 95%

Format : spin column

Operation time : 20 minutes

Elution volume : 20-50 µl

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Revised: 9/6/10

Introduction

The Gel/PCR DNA Fragments Extraction Kit was designed to recover or concentrate DNA fragments (100 bp→10 Kb) from agarose gel, PCR, or other enzymatic reactions. Chaotropic salt is used to dissolve agarose gel and denature enzymes. DNA fragments in the 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 entire procedure can be completed in 20 minutes and the eluted DNA is ready for use in PCR, Fluorescent or Radioactive Sequencing, Restriction Enzyme Digestion, DNA Labeling and Ligation. For users who require a higher recovery from small base pair DNA fragments (50-200 bp) or large base pair DNA fragments (> 8 Kb), see the order information below.

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 checked by electrophoresis.

Kit Contents

Name	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 (10 mM Tris-HCl, pH 8.5 at 25ºC)	1 ml	6 ml	30 ml
DF Column	4 pcs	100 pcs	300 pcs
2 ml Collection Tube	4 pcs	100 pcs	300 pcs

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

Order Information

Product Name	Package size	Cat. No.
Gel/PCR DNA Fragments Extraction Kit	100/300 preps	IB47020/030
Large DNA Fragments Extraction Kit (> 8Kb)	100/300 preps	IB47071/072
Small DNA Fragments Extraction Kit (50-200bp)	100/300 preps	IB47061/062
96-Well Gel/PCR DNA Extraction Kit	4/10 x 96 Wells	IB47040/050
Vacuum Manifold (Accessories)	1 SET	IB47500

Caution

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

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

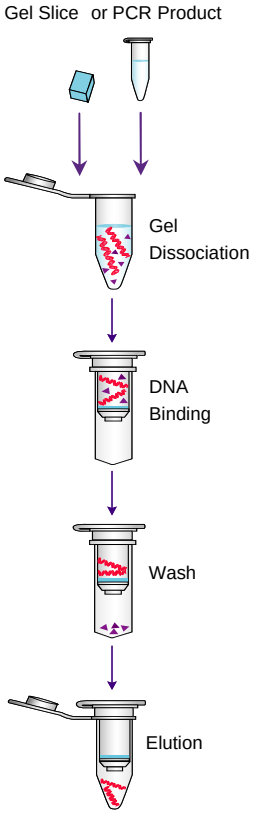
Additional requirements

microcentrifuge tubes, absolute ethanol

Gel Extraction Protocol

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

Step 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 (TAE buffer is recommended for gel formation). • Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube. • Add **500 µl of DF Buffer** to the sample and mix by vortex. • Incubate at 55-60ºC for 10-15 minutes or until the gel slice has been completely dissolved. During incubation, invert the tube every 2–3 minutes. • Cool the dissolved sample mixture to room temperature.
Step 2 DNA Binding	• Place the **DF Column** in a **2 ml Collection Tube**. • Transfer 800 µl of the sample mixture from the previous step 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** (If the sample mixture is more than 800 µl, repeat the DNA Binding Step).
Step 3 Wash	• Add **400 µl of W1 Buffer** into the **DF Column**. • Centrifuge at 14-16,000 x g for 30 seconds and then discard the flow-through. • Place the **DF Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (ethanol added) into the **DF Column** and let stand for 1 minute. • Centrifuge at 14-16,000 x g for 30 seconds and 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 4 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 2 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. • Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.



Gel Extraction (Sequencing) Protocol

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

Step 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 (TAE buffer is recommended for gel formation). • Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube. • Add **500 µl of DF Buffer** to the sample and mix by vortex. • Incubate at 55-60ºC for 10-15 minutes or until the gel slice has been completely dissolved. During incubation, invert the tube every 2–3 minutes. • Cool the dissolved sample mixture to room temperature.
Step 2 DNA Binding	• Place the **DF Column** in a **2 ml Collection Tube**. • Transfer 800 µl of the sample mixture from Step 1 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** (If the sample mixture is more than 800 µl, repeat the DNA Binding Step).
Step 3 Wash	• Add **600 µl of Wash Buffer** (ethanol added) into the **DF Column** and let stand for 1 minute. • Centrifuge at 14-16,000 x g for 30 seconds and then discard the flow-through. • Place the **DF Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (ethanol added) into the **DF Column** and let stand for 1 minute. • Centrifuge at 14-16,000 x g for 30 seconds and then discard the flow-through. • Place the **DF Column** back in the **2 ml Collection Tube**. • Centrifuge at 14-16,000 x g again for 3 minutes to dry the column matrix.
Step 4 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 2 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. • Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.

PCR Clean Up Protocol

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

Step 1 Sample Prep.	• Transfer up to 100 µl of a 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 2 DNA Binding	• Place a **DF Column** in a **2 ml Collection Tube**. • Transfer the sample mixture from step 1 to the **DF Column** and 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**.
Step 3 Wash	• Add **600 µl of Wash Buffer** (ethanol added) into the center of the **DF Column** and let stand for 1 minute. • 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 again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 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 2 minutes or until the **Elution Buffer** or TE is completely absorbed by the matrix. • Centrifuge for 2 minutes at 14-16,000 x g to elute the purified DNA.

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 the DNA fragments are larger than 10 Kb, use preheated Elution Buffer (60-70ºC) in the Elution Step to improve the elution efficiency.
Eluted DNA doesn't perform well in downstream applications.	**Residual ethanol contamination** • Following the 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.