For Research Use Only

IBI SCIENTIFIC

INSTRUCTION MANUAL

Small DNA Fragments Extraction Kit

IB47060 (4 Preparation Kit)
IB47061 (100 Preparation Kit)
IB47062 (300 Preparation Kit)

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

Recovery: up to 95%

Format: spin column

Time: within 10 minutes

Elution volume: 20-50 µl

Storage: dry at room temperature (15-25°C)

Introduction

The Small DNA Fragments Extraction Kit was designed to recover or concentrate DNA fragments (40-200 bp) 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. 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 cleanup. 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 Small 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	IB47060	IB47061	IB47062
SD Buffer	3 ml	80 ml	240 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer[1] (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
SD Columns	4	100	300
2 ml Collection Tubes	4	100	300

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

Plasmid DNA Purification		
Product	Package Size	Catalogue Number
I-Blue Mini Plasmid Kit	100/300 preps	IB47171/172
I-Blue Mini Plasmid Kit	25 preps	IB47181
I-Blue Mini Plasmid Kit (Endotoxin Free)	25 preps	IB47191
Fast Ion Plasmid Midi Kit	25 preps	IB47111
Fast Ion Plasmid Midi Kit (Endotoxin Free)	25 preps	IB47113
Fast Ion Plasmid Maxi Kit	10/25 preps	IB47121/122
Fast Ion Plasmid Midi Kit (Endotoxin Free)	10/25 preps	IB47124/125
96-Well Plasmid Kit	4/10 x 96 preps	IB47151/152

Post Reaction DNA Purification		
Product	Package Size	Catalogue Number
Gel/PCR DNA Fragments Extraction Kit	100/300 preps	IB47020/030
Small DNA Fragments Extraction Kit	100/300 preps	IB47061/062
96-Well Gel/PCR DNA Extraction Kit	4/10 x 96 preps	IB47040/050

CAUTION!

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

Small DNA Fragments Extraction Kit Functional Test Data

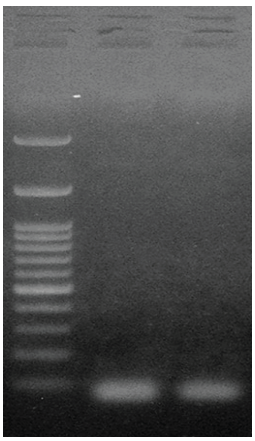

Figure 1. A 60 bp DNA fragment from E. coli 16S ribosomal DNA (1 µg) was extracted from a PCR product using the Small DNA Fragments Extraction Kit. The recovered DNA concentration was determined by spectrophotometer and analyzed by electrophoresis on a 1.5% agarose gel. Lane 1: Unpurified 60 bp DNA fragment (1 µg) Lane 2: Purified 60 bp DNA fragment (0.71 µg) M = 1 Kb DNA Ladder

DNA Conc.	260/280	Elution Volume	Total Yield	Recovery
14.20 µg/ml	1.73	50 µl	0.71 µg	71 %

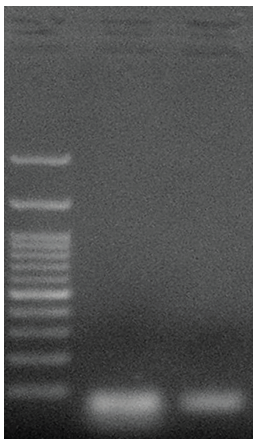

Figure 2. A 60 bp DNA fragment from E. coli 16S ribosomal DNA (1 µg) was extracted from gel using the Small DNA Fragments Extraction Kit. The recovered DNA concentration was determined by spectrophotometer and analyzed by electrophoresis on a 1.5% agarose gel. Lane 1: Unpurified 60 bp DNA fragment (1 µg) Lane 2: Purified 60 bp DNA fragment (0.60 µg) M = 1 Kb DNA Ladder

DNA Conc.	260/280	Elution Volume	Total Yield	Recovery
12.00 µg/ml	1.73	50 µl	0.60 µg	60%

Small DNA Fragments Gel Extraction Protocol

IMPORTANT BEFORE USE!

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

Gel Dissociation	• Excise the agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. NOTE: Use only TAE buffer for gel formation. • Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube. • Add 500 µl of SD Buffer to the sample and mix by 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 SD Column in a 2 ml Collection Tube. • Transfer 800 µl of the sample mixture to the SD Column then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the SD 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 SD Column. Or for sequencing following gel extraction add 600 µl of W1 Buffer into the SD Column and let stand for 1 minute. • Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. • Place the SD Column back in the 2 ml Collection Tube. • Add 600 µl of Wash Buffer (make sure ethanol was added) into the SD Column and let stand for 1 minute. • Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. • Place the SD 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 3 DNA Elution	• Transfer the dried SD 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 absorbed by the matrix. • Centrifuge at 14-16,000 x g for 2 minutes to elute the purified DNA.

Small DNA Fragments Gel Extraction Protocol

IMPORTANT BEFORE USE!

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

Sample Prep.	• Transfer up to 100 µl of a reaction product to a 1.5 ml microcentrifuge tube. • Add 5 volumes of SD Buffer to 1 volume of the sample and mix by vortex.
Step 1 DNA Binding	• Place a SD Column in a 2 ml Collection Tube. • Transfer the sample mixture to the SD Column and centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the SD 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 SD Column. • Let stand for 1 minute then centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the SD 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 3 DNA Elution	• Transfer the dried SD 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 absorbed by the matrix. • Centrifuge at 14-16,000 x g for 2 minutes to elute the purified DNA.

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 SD Column.
DNA doesn't perform well in downstream applications	**Residual ethanol contamination** • Following the Wash Step, dry the SD 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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7445 Chavenelle Road • Dubuque, IA 52002
800-253-4942 • (563) 690-0484 • info@ibisci.com • IBISCI.com