Small 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
Time: within 10 minutes
Elution volume: 20-50 µl
Storage: dry at room temperature (15-25ºC)

Geneaid

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

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	DF001	DF101	DF301
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

Order Information

Post Reaction DNA Purification		
Product	Package Size	Cat. Number
GenepHlow™ Gel Extraction Kit	100/300 preps	DFG100/300
GenepHlow ™ PCR Cleanup Kit	100/300 preps	DFC100/300
GenepHlow ™ Gel/PCR Kit	100/300 preps	DFH100/300
GenepHlow ™ DNA Cleanup Maxi Kit	10/25 preps	DFM010/025
Small DNA Fragments Extraction Kit	100/300 preps	DF101/301
Presto™ Max Gel/PCR Kit (Large DNA Fragments)	100/300 preps	DFL100/300
Presto™ 96 Well PCR Cleanup Kit	4/10 x 96 preps	96DFH04/10
Presto™ PCR Cleanup Kit 96 Well Binding Plate	10 plates	96DBP01
DNA Cleanup Kit	100/300 preps	DP100/300
G-25 Gel Filtration Desalting Column	50 rxns	CG025
G-50 Gel Filtration Dye Terminator Removal Column	50 rxns	CG050
96-Well G-50 Gel Filtration Plate	4/10 x 96 rxns	CGP04/10
Gel Extraction Tool	25 pcs	GXT025

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

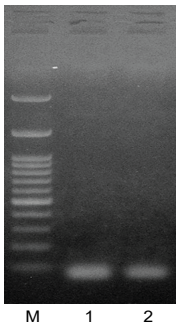

Figure 1. A 60 bp DNA fragment from *E. coli* 16S ribosomal DNA (1 µg) was extracted from a PCR product using the Small DNA Fragments Extraction Kit. The recovered DNA concentration was determined by spectrophotometer and analyzed by electrophoresis on a 1.5% agarose gel.
Lane 1: Unpurified 60 bp DNA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

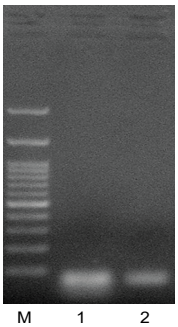

Figure 2. A 60 bp DNA fragment from *E. coli* 16S ribosomal DNA (1 µg) was extracted from gel using the Small DNA Fragments Extraction Kit. The recovered DNA concentration was determined by spectrophotometer and analyzed by electrophoresis on a 1.5% agarose gel.
Lane 1: Unpurified 60 bp DNA fragment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

IMPORTANT BEFORE USE

• Add absolute ethanol (see the bottle label for volume) to the 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

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. • Use ≤2% agarose gel to ensure optimal dissolution efficiency and DNA yield. • 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 SD Column.
Eluted 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 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.