

Large DNA Fragments Extraction Kit

For research use only

Sample	: agarose gel
Recovery	: up to 85%
Format	: spin column
Operation time	: 45 minutes

Geneaid



www.geneaid.com

Introduction

The Large DNA Fragments Extraction Kit was designed to recover or concentrate DNA fragments (> 8 Kb) from agarose gel in 4 easy steps. Salts and enzymes can be effectively removed from the reaction mixture without phenol extraction. Typically, recoveries are up to 85% for Gel Extraction. The entire procedure can be completed in 45 minutes and the DNA is ready for use in PCR, Fluorescent or Radioactive Sequencing, Restriction Enzyme Digestion, DNA Labeling and Ligation.

Quality Control

The quality of the Large DNA Fragments Extraction Kit is tested on a lot-to-lot basis by isolating DNA fragments of various sizes from agarose gel. The purified DNA is checked by electrophoresis.

Kit Contents

Name	DF002	DF102	DF302
LD Buffer*	100 µl	3 ml	10 ml
Wash Buffer** (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml + 25 ml (200 ml) (100 ml)
Resuspension Buffer (10 mM Tris-HCl, pH 8.5 at 25°C)	1 ml	6 ml	30 ml
LD Column	4 pcs	100 pcs	300 pcs

Order Information

Product Name	Package Size	Cat. No.
Gel/PCR DNA Fragments Extraction Kit	100/300 preps	DF100/300
Small DNA Fragments Extraction Kit (optimized for 50-200 bp)	100/300 preps	DF101/301
Large DNA Fragments Extraction Kit (optimized for > 8 Kb)	100/300 preps	DF102/302
96-Well Gel/PCR DNA Extraction Kit	2/4/10 X 96 Wells	DFP02/04/10
Vacuum Manifold (Accessories)	1 SET	ZVF01

*If precipitates have formed in the LD Buffer, warm the buffer in a 37°C water bath to dissolve.

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

Caution

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

1.5 ml microcentrifuge tubes, absolute ethanol

Gel Extraction Protocol

If precipitates have formed in the LD Buffer, warm the buffer in a 37°C water bath to dissolve.

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

Step 1 Gel Processing	<ul style="list-style-type: none">Excise the agarose gel slice (300 mg) containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice.Place an LD Column in a new 1.5 ml microcentrifuge tube and transfer the gel slice to the LD Column.Centrifuge at 2,000 x g for 15 minutes.Discard the LD Column and add ddH₂O to the flow-through in the 1.5 ml microcentrifuge tube until the total volume is approximately 100 µl.Add 10 µl of LD Buffer to the sample and mix by vortex.
Step 2 DNA Precipitation	<ul style="list-style-type: none">Add 300 µl of absolute ethanol to the sample and mix by vortex.Place the tube on ice for 5 minutes.Centrifuge at 14-16,000 x g for 20 minutes.
Step 3 DNA Wash	<ul style="list-style-type: none">Carefully remove the supernatant.Add 600 µl of Wash Buffer to wash the DNA pellet.Centrifuge at 14-16,000 x g for 1 minute.Discard the supernatant completely.
Step 4 DNA Resuspension	<ul style="list-style-type: none">Add 20-50 µl of Resuspension Buffer to resuspend the DNA pellet completely.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	<p>Gel percentage is too high</p> <ul style="list-style-type: none">0.8-1.0% agarose gel is recommended. <p>Insufficient centrifuge in Step 1</p> <ul style="list-style-type: none">Increase centrifuge time or the centrifuge speed (do not exceed 5,000 x g).
Low A260/A230	<ul style="list-style-type: none">In the wash step, repeat the 600 µl of Wash Buffer addition and let stand for 1 minute.