Large DNA Fragments Extraction Kit

For research use only

Sample : agarose gel

Recovery : up to 85%

Format : spin column

Operation time : 45 minutes

www.ibisci.com

Revised: 9/6/10

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	IB47070	IB47071	IB47072
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 25ºC)	1 ml	6 ml	30 ml
LD Column	4 pcs	100 pcs	300 pcs

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

**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

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 37ºC water bath to dissolve.
- Add absolute ethanol (see the bottle label for details) to the Wash Buffer prior to initial use.

Step 1 Gel Processing	• 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 ddH_20 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	• 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	• 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	• Add **20-50 µl of Resuspension Buffer** to resuspend the DNA pellet completely.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	**Gel percentage is too high** • 0.8-1.0% agarose gel is recommended.
	Insufficient centrifuge in Step 1 • Increase centrifuge time or the centrifuge speed (do not exceed 5,000 x g).
Low A260/A230	• In the wash step, repeat the 600 µl of Wash Buffer addition and let stand for 1 minute.