HiYield™ 96-Well Gel/PCR DNA Extraction Kit

Cat. No.:	YDF96B-2	YDF96B-4	YDF96B-10
Product Name:	HiYield™ 96-Well Gel/PCR DNA Extraction Kit		
Reactions:	2	4	10
Format:	96-Well Plates		
DNA Size:	50bp-10kb		
Operation:	Centrifuge or Vacuum		
Operation Time:	40 Minutes for Gel Extraction / 30 Minutes for PCR Cleanup		

Introduction

HiYield™ 96-Well Gel/PCR DNA Extraction Kit provides a high-throughput, rapid and economical method to purify DNA fragments (50 bp~10 kb) from agarose gels, PCR or other enzymatic reaction. The unique dual purpose application and high yield 96-well plate makes this kit exceptional value. Salts, enzymes and unincorporated nucleotides are effectively removed from reactions mixtures without toxic phenol extraction and alcohol precipitation. The entire procedure can be completed in 40 minutes and the eluted DNA is ready to use in restriction enzyme digestions, ligation, PCR and sequencing reactions.

Features

Up to 95% recovery of ready-to-use DNA with high-throughput extraction.
Unique dual purpose application(Gel/PCR DNA Extraction).
Without phenol extraction or alcohol precipitation.

Applications

Purified DNA is ready for direct use in PCR, DNA Sequencing, DNA Library Screening and Analysis, Restriction Enzyme Digestion, DNA Labeling, Preparation of PCR Probes for Microarrays, Ligation and Transformation.

Quality Control

The quality of HiYield™ 96-Well Gel/PCR DNA Extraction Kit is tested on a lot lot basis. The efficiency of DNA recovery is tested by isolation of DNA fragments of various sizes from either aqueous solutions or agarose gel. The purified DNA is checked by agarose gel analysis.

Contents

ITEM	YDF96B-2	YDF96B-4	YDF96B-10
DNA Binding Plate	2 plates	4 plates	10 plates
Adhesive Film	4 pcs	8 pcs	20 pcs
350µl Collection Plate	2 plates	4 plates	10 plates
Binding Buffer	80 ml	120 ml	320 ml
W1 Buffer	60 ml	130 ml	260 ml
Wash Buffer(concentrated)	12.5 ml	25 ml	50 ml
Elution Buffer	30 ml	30 ml	60 ml

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

Caution

Buffers contain irritant agents. 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.

PCR Cleanup Centrifuge Protocol

Additional Requirements:

Centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol.

Things Before Starting:

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

Step1 DNA Binding	★Transfer up to 50 µl of PCR product to a 2 ml collection plate. ★Add **250 µl of Binding Buffer** to each well and mix by pipetting. ★Place a **DNA Binding Plate** on a new 2 ml collection plate. ★Transfer the sample mixture to each well of the **DNA Binding Plate**. ★Centrifuge for 5 minutes at 1,000 x g. ★Discard the flow-through and place the **DNA Binding Plate** back on the 2 ml collection plate.
Step 2 Wash	★Add **250 µl of W1 Buffer** into each well of the **DNA Binding Plate**. ★Centrifuge for 5 minutes at 1,000 x g. ★Add **250 µl of Wash Buffer** (ethanol added) into each well of the **DNA Binding Plate** to wash again and let stand for 1 minute. ★Centrifuge for 5 minutes at 1,000 x g. ★Discard the flow-through and press the **DNA Binding Plate** on an absorbent material to blot out the excess liquid from the bottom of the plate. ★Place the **DNA Binding Plate** back on the 2 ml collection plate. ★Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue.
Step 3 DNA Elution	★Transfer the **DNA Binding Plate** to a **350 µl Collection Plate**. ★Add **50 µl of Elution Buffer** or TE to the center of each well. ★Let stand for 2 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA.

PCR Cleanup Vacuum Protocol

Additional Requirements:

Multi-well plate vacuum manifold, (ex: HiYield™ 96-Well Vacuum Manifold), centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol.

Things Before Starting:

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

Step1 DNA Binding	★Transfer up to 50 µl of PCR product to a 2 ml collection plate. ★Add **250 µl of Binding Buffer** to each well and mix by pipetting. ★Place a new 2 ml collection plate on the base of the vacuum manifold and place a **DNA Binding Plate** on top of the vacuum manifold. ★Transfer the sample mixture to each well of the **DNA Binding Plate**. ★Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied.
Step 2 Wash	★Turn off the vacuum pump and add **250 µl of W1 Buffer** to each well of the **DNA Binding Plate**. ★Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied. ★Turn off the vacuum pump and add **250 µl of Wash Buffer** (ethanol added) to each well of the **DNA Binding Plate** to wash again and let stand for 1 minute. ★Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied. ★Turn off the vacuum pump and press the **DNA Binding Plate** on an absorbent material to blot out the excess liquid from the bottom of the plate.
Step 3 DNA Elution	★Place the **DNA Binding Plate** back on the 2 ml collection plate. ★Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue. ★Transfer the **DNA Binding Plate** to a **350 µl Collection Plate**. ★Add **50 µl of Elution Buffer** or TE to the center of each membrane. ★Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA.

Gel Extraction Centrifuge Protocol

Additional Requirements:

Centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol.

Things Before Starting:

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

Step1 DNA Binding	★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 50 mg of the gel slice to a 2 ml collection plate. ★Add **250 µl of Binding Buffer** to each well and mix by pipetting. ★Incubate at 55-60ºC for 10 minutes or until the gel slice has been completely dissolved. ★Place a **DNA Binding Plate** on a new 2 ml collection plate. ★Transfer the sample mixture to each well of the **DNA Binding Plate**. ★Centrifuge for 5 minutes at 1,000 x g. ★Discard the flow-through and place the **DNA Binding Plate** back on the 2 ml collection plate.
Step 2 Wash	★Add **250 µl of W1 Buffer** into each well of the **DNA Binding Plate**. ★Centrifuge for 5 minutes at 1,000 x g. ★Add **250 µl of Wash Buffer** (ethanol added) into each well of the **DNA Binding Plate** to wash again and let stand for 1 minute. ★Centrifuge for 5 minutes at 1,000 x g. ★Discard the flow-through and press the **DNA Binding Plate** on an absorbent material to blot out the excess liquid from the bottom of the plate. ★Place the **DNA Binding Plate** back on the 2 ml collection plate. ★Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue.
Step 3 DNA Elution	★Transfer the **DNA Binding Plate** to a **350 µl Collection Plate**. ★Add **50 µl of Elution Buffer** or TE to the center of the membrane. ★Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA.

Gel Extraction Vacuum Protocol

Additional Requirements:

Multi-well plate vacuum manifold, (ex: HiYield™ 96-Well Vacuum Manifold), centrifugation system for 96-well plates, 2 ml collection plates absolute ethanol.

Things Before Starting:

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Step 3 **DNA Elution**	★Place the **DNA Binding Plate** back on the 2 ml collection plate. ★Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue. ★Transfer the **DNA Binding Plate** to a **350 µl Collection Plate**. ★Add **50 µl of Elution Buffer** or TE to the center of each membrane. ★Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ★Centrifuge for 5 minutes at 1,000 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 50 mg of gel slice, separate it into multiple wells. →Raise incubation temperature to 60ºC and extend incubation time. **Incorrect DNA Elution Step** →Ensure that the Elution Buffer is added and absorbed to the center of each well. **Incomplete DNA Elution** →If the sizes of the DNA fragments are larger than 10 Kb, use preheated Elution Buffer (60-70°C) in the Elution Step to improve the elution efficiency.
Eluted DNA does not perform well in downstream applications	**Residual ethanol contamination** →Following the wash step, dry the DNA Binding Plate with additional centrifugation at full speed for 5 minutes or incubate at 60ºC for 5 minutes. **DNA was denatured (a smaller band appeared on the gel analysis)** →Incubate the eluted DNA at 95ºC for 2 minutes, and then cool down slowly to re-anneal the denatured DNA.