

# 96-Well Gel/PCR DNA Extraction Kit

*For research use only*

- Sample** : up to 50 µl of PCR product or other enzymatic reactions  
up to 50 mg of agarose gel slice
- Format** : 96-well plates
- DNA size** : 100 bp→10 Kb
- Operation** : centrifuge/vacuum manifold
- Recovery** : up to 85% for Gel Extraction  
up to 90% for PCR Clean up
- Operation time** : 40 minutes for Gel Extraction  
30 minutes for PCR Clean up

**Geneaid**



www.geneaid.com

## Introduction

The 96-Well Gel/PCR DNA Extraction Kit provides a high-throughput method to recover or concentrate DNA fragments from agarose gel, PCR, or other enzymatic reactions. Chaotropic salt is used to denature enzymes and in this condition, DNA fragments are bound by the glass fibre matrix (1) of each well of the plate. Once any contaminants have been removed, using the Wash Buffer (containing ethanol), the purified DNA is eluted by a low salt Elution Buffer or TE. Salts, enzymes and unincorporated nucleotides are effectively removed from reaction mixtures without toxic phenol extraction or alcohol precipitation. The entire procedure can be completed in 30-40 minutes and the eluted DNA is ready for use in Restriction Enzyme Digestion, Ligation, PCR, and Sequencing Reactions.

## Quality Control

The quality of the 96-Well Gel/PCR DNA 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 checked by electrophoresis.

### Kit Contents

Name	DFP02	DFP04	DFP10
Binding Buffer	80 ml	120 ml	320 ml
W1 Buffer	60 ml	130 ml	130 ml x 2
Wash Buffer* (Add Ethanol)	12.5 ml (50 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	30 ml	30 ml	60 ml
DNA Binding Plate	2 pcs	4 pcs	10 pcs
0.35 ml Collection Plate	2 pcs	4 pcs	10 pcs
Adhesive film	4 pcs	8 pcs	20 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

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

## 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

## 96-Well Gel/PCR DNA Extraction Kit (PCR Clean up) Centrifuge Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol

Step1 DNA Binding	<ul style="list-style-type: none"> <li>● Transfer up to 50 <math>\mu</math>l of PCR product to a 2 ml collection plate.</li> <li>● Add <b>250 <math>\mu</math>l of Binding Buffer</b> to each well and mix by pipetting.</li> <li>● Place a <b>DNA Binding Plate</b> on a new 2 ml collection plate.</li> <li>● Transfer the sample mixture to each well of the <b>DNA Binding Plate</b>.</li> <li>● Centrifuge for 5 minutes at 1,000 x g.</li> <li>● Discard the flow-through and place the <b>DNA Binding Plate</b> back on the 2 ml collection plate.</li> </ul>
Step 2 Wash	<ul style="list-style-type: none"> <li>● Add <b>250 <math>\mu</math>l of W1 Buffer</b> into each well of the <b>DNA Binding Plate</b>.</li> <li>● Centrifuge for 5 minutes at 1,000 x g.</li> <li>● Add <b>250 <math>\mu</math>l of Wash Buffer</b> (ethanol added) into each well of the <b>DNA Binding Plate</b> to wash again and let stand for 1 minute.</li> <li>● Centrifuge for 5 minutes at 1,000 x g.</li> <li>● Discard the flow-through and press the <b>DNA Binding Plate</b> on an absorbent material to blot out the excess liquid from the bottom of the plate.</li> <li>● Place the <b>DNA Binding Plate</b> back on the 2 ml collection plate.</li> <li>● Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue.</li> </ul>
Step 3 DNA Elution	<ul style="list-style-type: none"> <li>● Transfer the <b>DNA Binding Plate</b> to a <b>0.35 ml Collection Plate</b>.</li> <li>● Add <b>50 <math>\mu</math>l of Elution Buffer</b> or TE to the center of each well.</li> <li>● Let stand for 2 minutes or until the <b>Elution Buffer</b> or TE is absorbed by the matrix.</li> <li>● Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA.</li> </ul>

## 96-Well Gel/PCR DNA Extraction Kit (PCR Clean up) Vacuum Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: multi-well plate vacuum manifold, centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol

Step1 DNA Binding	<ul style="list-style-type: none"> <li>● Transfer up to 50 <math>\mu</math>l of PCR product to a 2 ml collection plate.</li> <li>● Add <b>250 <math>\mu</math>l of Binding Buffer</b> to each well and mix by pipetting.</li> <li>● Place a new 2 ml collection plate on the base of the vacuum manifold and place a <b>DNA Binding Plate</b> on top of the vacuum manifold.</li> <li>● Transfer the sample mixture to each well of the <b>DNA Binding Plate</b>.</li> <li>● Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied.</li> </ul>
Step 2 Wash	<ul style="list-style-type: none"> <li>● Turn off the vacuum pump and add <b>250 <math>\mu</math>l of W1 Buffer</b> to each well of the <b>DNA Binding Plate</b>.</li> <li>● Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied.</li> <li>● Turn off the vacuum pump and add <b>250 <math>\mu</math>l of Wash Buffer</b> (ethanol added) to each well of the <b>DNA Binding Plate</b> to wash again and let stand for 1 minute.</li> <li>● Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied.</li> <li>● Turn off the vacuum pump and press the <b>DNA Binding Plate</b> on an absorbent material to blot out the excess liquid from the bottom of the plate.</li> <li>● Place the <b>DNA Binding Plate</b> back on the 2 ml collection plate.</li> <li>● Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue.</li> </ul>
Step 3 DNA Elution	<ul style="list-style-type: none"> <li>● Transfer the DNA Binding Plate to a 0.35 ml Collection Plate.</li> <li>● Add <b>50 <math>\mu</math>l of Elution Buffer</b> or TE to the center of each membrane.</li> <li>● Let stand for 3 minutes or until the <b>Elution Buffer</b> or TE is absorbed by the matrix.</li> <li>● Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA.</li> </ul>

## 96-Well Gel/PCR DNA Extraction Kit (Gel Extraction) Centrifuge Protocol

- Add absolute ethanol to (see the bottle label for volume) the Wash Buffer prior to initial use
- Additional requirements: centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol

Step1 DNA Binding	<p>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).</p> <ul style="list-style-type: none"> <li>● Transfer up to 50 mg of the gel slice to a 2 ml collection plate.</li> <li>● Add <b>250 µl of Binding Buffer</b> to each well and mix by pipetting.</li> <li>● Incubate at 55-60°C for 10 minutes or until the gel slice has been completely dissolved.</li> <li>● Place a <b>DNA Binding Plate</b> on a new 2 ml collection plate.</li> <li>● Transfer the sample mixture to each well of the <b>DNA Binding Plate</b>.</li> <li>● Centrifuge for 5 minutes at 1,000 x g.</li> <li>● Discard the flow-through and place the <b>DNA Binding Plate</b> back on the 2 ml collection plate.</li> </ul>
Step 2 Wash	<ul style="list-style-type: none"> <li>● Add <b>250 µl of W1 Buffer</b> into each well of the <b>DNA Binding Plate</b>.</li> <li>● Centrifuge for 5 minutes at 1,000 x g.</li> <li>● Add <b>250 µl of Wash Buffer</b> (ethanol added) into each well of the <b>DNA Binding Plate</b> to wash again and let stand for 1 minute.</li> <li>● Centrifuge for 5 minutes at 1,000 x g.</li> <li>● Discard the flow-through and press the <b>DNA Binding Plate</b> on an absorbent material to blot out the excess liquid from the bottom of the plate.</li> <li>● Place the <b>DNA Binding Plate</b> back on the 2 ml collection plate.</li> <li>● Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue.</li> </ul>
Step 3 DNA Elution	<ul style="list-style-type: none"> <li>● Transfer the <b>DNA Binding Plate</b> to a <b>0.35 ml Collection Plate</b>.</li> <li>● Add <b>50 µl of Elution Buffer</b> or TE to the center of the membrane.</li> <li>● Let stand for 3 minutes or until the <b>Elution Buffer</b> or TE is absorbed by the matrix.</li> <li>● Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA.</li> </ul>

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- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
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Step1 DNA Binding	<p>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).</p> <ul style="list-style-type: none"> <li>● Transfer up to 50 mg of the gel slice to a 2 ml collection plate.</li> <li>● Add <b>250 µl of Binding Buffer</b> to each well and mix by pipetting.</li> <li>● Incubate at 55-60°C for 10 minutes or until the gel slice has been completely dissolved.</li> <li>● Place a new 2 ml collection plate on the base of the vacuum manifold and place a <b>DNA Binding Plate</b> on top of the vacuum manifold.</li> <li>● Transfer the sample mixture to each well of the <b>DNA Binding Plate</b>.</li> <li>● Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied.</li> </ul>
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## Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	<b>Gel slice did not dissolve completely</b> <ul style="list-style-type: none"><li>● The Gel slice was too big. If using more than 50 mg of gel slice, separate it into multiple wells.</li><li>● Raise incubation temperature to 60°C and extend incubation time.</li></ul>
	<b>Incorrect DNA Elution Step</b> <ul style="list-style-type: none"><li>● Ensure that the Elution Buffer is added and absorbed to the center of each well.</li></ul>
	<b>Incomplete DNA Elution</b> <ul style="list-style-type: none"><li>● 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.</li></ul>
Eluted DNA does not perform well in downstream applications	<b>Residual ethanol contamination</b> <ul style="list-style-type: none"><li>● Following the wash step, dry the DNA Binding Plate with additional centrifugation at full speed for 5 minutes or incubate at 60°C for 5 minutes.</li></ul>
	<b>DNA was denatured (a smaller band appeared on the gel analysis)</b> <ul style="list-style-type: none"><li>● Incubate the eluted DNA at 95°C for 2 minutes, and then cool down slowly to re-anneal the denatured DNA.</li></ul>