

GEL/PCR Purification Kit

Description

The DE-001 Gel/PCR Purification Kit is designed to recover or concentrate DNA Fragments (50bp-10Kb) from agarose gel, PCR or other enzymatic reaction. The unique dual purpose application and high yield DNA column make this kit exceptional value.

Features

- With simple steps, quick and easy to use.
- Highly pure DNA (suitable for PCR).
- No phenol/chloroform extraction and ethanol precipitation required.

Applications

- PCR
- Fluorescent or Radioactive Sequencing
- Restriction Digestion
- DNA Labeling
- Ligation and Transformation

Sample Source

Up to 300mg agarose gel slice

Up to 100 μ l PCR product or other enzymatic reaction

DNA Size Range

100bp ~20kb

Binding Capacity of Spin Filter

20 ~100 μ l of PCR product

Recovery Rate

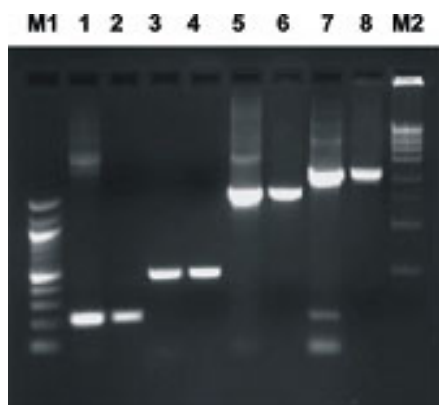
80 ~90% for GEL extraction

90~95% for PCR Clean-Up

Handling Time

10 minutes for PCR clean up

20 minutes for GEL extraction



The Quality of DNA After Purification

DNA fragments before and after extraction with the GEL/PCR Purification Mini Kit

Lane 1, 3, 5, 7 before extraction: 200bp, 500bp, 2Kb, 3Kb.

Lane 2, 4, 6, 8 after extraction: 200bp, 500bp, 2Kb, 3Kb

M1: 100bp DNA Ladder

M2: 1Kb DNA Ladder

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Procedure

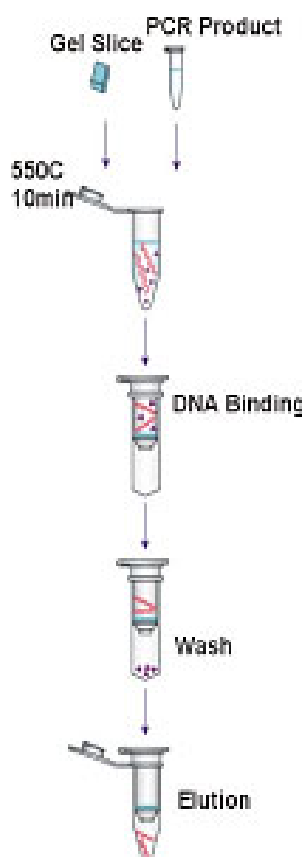
The method uses a chaotropic salt, guanidine thiocyanate to dissolve the agarose gel and denature enzymes. The DNA fragment in the chaotropic salt is bond to the glass fiber matrix of the spin column. After washing off the contaminants, the purified DNA fragments are eluted by a low salt elution buffer or water. Salts, enzymes and unincorporated nucleotides can be effectively removed from reaction mixture without phenol extraction and alcohol precipitation.

Storage Conditions

Stable for 1 year at room temperature.
The kit is shipped at ambient temperature.

Ordering Information

Cat. No.	Product Name	Size	Kit Components	Store at
DE-001	GEL/PCR Purification Kit	100 preps.	FSDF Buffer Wash Buffer (conc.) Elution Buffer FSDF columns 2 ml Collection tubes	Storee at room temperature for 1 year.
DE-002	GEL/PCR Purification Kit	300 preps.	FSDF Buffer Wash Buffer (conc.) Elution Buffer FSDF columns 2 ml Collection tubes	Storee at room temperature for 1 year.



Distributed by:



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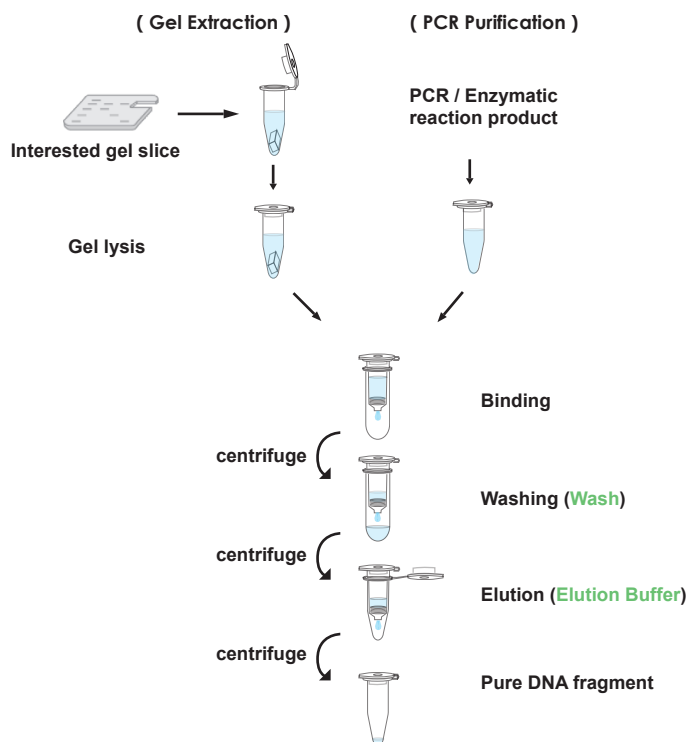
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36 Terry Drive
Trevose, PA 19048 - USA

Kit Contents:	DE-001 (100 preps)	DE-002 (300 preps)
FSDF Buffer	80 ml	240 ml
Wash Buffer*	25 ml	50 ml
Elution Buffer	6 ml	30 ml
FSDF Column	100 pcs	300 pcs
2 ml Collection Tube	100 pcs	300 pcs

*Add 100 ml / 200 ml ethanol (96-100%) to Wash Buffer when first open.



Specification:

Sample: up to 300 mg of agarose gel
 up to 100 µl of reaction solution
 Recovery: 70% ~ 85% for Gel extraction
 90% ~ 95% for PCR clean-up
 Operation time: 20 min
 Elution volume: 40 µl

Important Notes:

1. Buffers provided in this kit contain irritants.
Wear gloves and lab coat when handling these buffers.
2. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

• Gel Extraction Protocol:

HINT: Prepare a 55 °C dry bath or water bath for step 4.

1. Excise the the agarose gel with a clean scalpel.
 • **Remove the extra agarose gel to minimize the size of the gel slice.**
2. Transfer up to 300 mg of the gel slice into a microcentrifuge tube.(not provided).
 • **The maximum volume of the gel slice is 300mg.**
3. Add 500 µl of FSDF Buffer to the sample and mix by vortexing.
 • **For > 2% agarose gels, add 1000 µl of FSDF Buffer.**
4. Incubate at 55 °C for 10-15 minutes and vortex the tube every 2-3 min until the gel slice dissolved completely.
 • **During incubation, interval vortex can accelerate the gel dissolved.**
 • **Make sure that the gel slice has been dissolved completely before proceed the next step.**
5. Cool down the sample mixture to room temperature. And place a FSDF Column in a Collection Tube.
6. Transfer 800 µl of the sample mixture to FSDF Column.
 Centrifuge for 30 seconds then discard the flow-through.
 • **If the sample mixture is more than 800 µl, repeat this step for the rest sample mixture.**
7. Add 750 µl of Wash Buffer (ethanol added) to the FSDF Column.
 Centrifuge for 30 seconds then discard the flow-through.
 • **Make sure that ethanol (96-100 %) has been added into Wash Buffer when first open.**

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8. Centrifuge again for an additional 3 minutes to dry the column.
• **Important step ! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.**

9. Place the FSDF Column to a new microcentrifuge tube (not provided).

10. Add 40 µl of Elution Buffer or ddH₂O to the membrane center of the FSDF Column.
Stand the FSDF Column for 2 min.

• **Important step !** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.

• **Important :** Do not elute the DNA using less than suggested volume (40µl). It will lower the final yield.

11. Centrifuge for 2 min to elute the DNA.

12. Store the DNA at 4 °C or -20 °C.

• **PCR Clean-Up Protocol:**

1. Transfer up to 100 µl of PCR product (excluding oil) and add 5 volumes of FSDF Buffer to a microcentrifuge tube(not provided) then mix by vortexing.

• **The maximum volume of PCR product is 100 µl (excluding oil). Do not exceed this limit. If PCR product is more than 100 µl, separate it into multiple tubes.**

2. Place a FSDF column into a Collection Tube.

3. Transfer the sample mixture to the FSDF Column. Centrifuge for 30 seconds then discard the flow-through.

4. Add 750 µl of Wash Buffer (ethanol added) to the FSDF Column.
Centrifuge for 30 seconds then discard the flow-through.

• **Make sure that ethanol (96-100 %) has been added into Wash Buffer when first open.**

5. Centrifuge again for an additional 3 minutes to dry the column.

• **Important step ! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.**

6. Place the FSDF Column to a new microcentrifuge tube (not provided).

7. Add 40 µl of Elution Buffer or ddH₂O to the membrane center of the FSDF Column.
Stand the FSDF Column for 2 min.

• **Important step !** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.

• **Important :** Do not elute the DNA using less than suggested volume (40µl). It will lower the final yield.

8. Centrifuge for 2 min to elute the DNA.

9. Store the DNA at 4 °C or -20 °C.

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• Troubleshooting

The gel slice is hard to dissolve:

The size of the gel slice is too large

- **If the gel slice is more than 300 mg, separate it into multiple tubes.**
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Low or none recovery of DNA fragment:

(For Gel Purification) The column is loaded with too much agarose gel

- **The maximum volume of the gel slice is 300 mg per column**

(For PCR Clean-Up) Apply more than 100 µl of PCR product

- **If PCR product is more than 100 µl, separate it into multiple tubes.**

Elution of DNA fragment is not efficient

- **Make sure the pH of Elution Buffer or ddH₂O is between 7.0-8.5.**
- **Make sure the elution solution has been completely absorbed by the membrane before centrifugation.**

The size of DNA fragment is larger than 5 Kb

- **Preheat the elution solution to 60°C before use.**
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Eluted DNA contains non-specific DNA fragment

(For Gel Purification) Contaminated scalpel

- **Using a clean scalpel.**

DNA fragment is denatured

- **Incubate eluted DNA at 95°C for 2 minutes, then cool down slowly to reanneal denatured DNA.**
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Poor performance in the downstream applications

Salt residue remains in eluted DNA fragment

- **Wash the column twice with Wash Buffer**

Ethanol residue remains in eluted DNA fragment

- **Make sure you have discarded the flow-through after washing with Wash Buffer and centrifuged for an additional 3 minutes.**
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