For general laboratory use

Gene Transfer Pioneers
(GTP)

DNA Gel Recovery Kit
(PCR Purification Kit)

Kit for 25 purifications

Catalogue Number: DR02025

Store the kit at +15 to +25°C.

Introduction

The **DNA Gel Recovery Kit (PCR Purification Kit)** was designed to recover or concentrate DNA fragments (70 bp-20 kb) from agarose gel, PCR, or other enzymatic reactions in one convenient product. Chaotropic salt is used to dissolve agarose gel and denature enzymes. DNA fragments in chaotropic salt are bound by the glass fiber matrix of the spin column. Contaminants are removed with a Wash Buffer (containing ethanol) and the purified DNA fragments are eluted by a low salt Elution Buffer or TE. Salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture, without phenol extraction or alcohol precipitation. Typically, recoveries are up to 90% for Gel Extraction and up to 95% for PCR Clean Up. The eluted DNA is ready for use in PCR, Fluorescent or Radioactive Sequencing, Restriction Enzyme Digestion, DNA Labeling and Ligation.

Quality Control

The quality of the **DNA Gel Recovery Kit (PCR Purification 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 analyzed by electrophoresis.

Kit Contents

All solutions are clear. If any solution contains a precipitate, do not use it. Instead, warm the solution at room temperature or in a 37°C water bath to dissolve the precipitate.

Vial	Label	Contents
1. Black Cap	DR Buffer	15 ml
2. Blue Cap	Wash Buffer	3 ml; add 12 ml ethanol
3. White Cap	Elution Buffer	10 ml
4. Colorless Bag	DNA Spin Column	25 Columns

Storage and Stability

Store the DNA Gel Recovery Kit (PCR Purification Kit) components at +15 to +25°C. Kit components are guaranteed to be stable date printed on the label.

Application: The kit is designed for the efficient and convenient isolation of DNA from agarose gel and PCR products from amplification reactions. Primers, mineral oil, salts, unincorporated nucleotides, and the thermostable DNA polymerase may inhibit subsequent enzymatic reactions (*e.g.,* labeling, sequencing or cloning of the PCR products). This kit is also recommended for the purification of cDNA.

In addition, nucleic acids from other modification reactions (*e.g.,* restriction endonuclease digests, alkaline-phosphatase treatment, or kinase reactions) can be purified using this kit. It can also be applied to concentrate dilute nucleic-acid solutions.

Preparation Time The entire DNA Gel Recovery Kit (PCR Purification Kit) method takes approx. 10 min.

Before You Begin: Preparation of Working Solutions

Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solution:

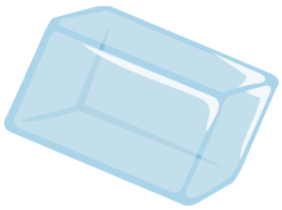
Content	Preparation	Storage/ Stability
Wash Buffer (Blue Cap)	Add 12 ml absolute ethanol to Wash Buffer. * Label bottle accordingly after adding ethanol.	Store at +15 to +25°C. Stable through expiration date printed on kit label.

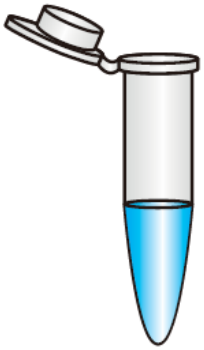
Caution

DF Buffer contains guanidine thiocyanate. During operation, always wear a lab coat, disposable gloves, and protective goggles.

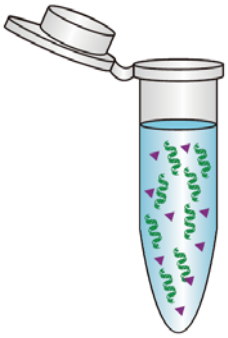
Quick Protocol Diagram

Gel Slice

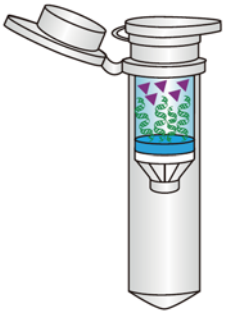




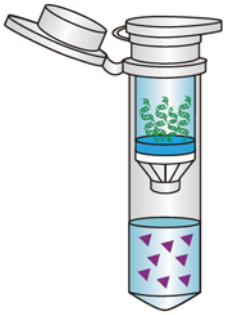
PCR Product



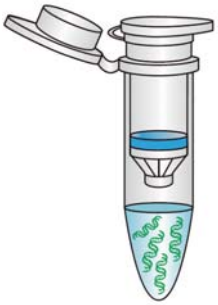
Lysis



DNA Binding



Wash



Elution

Purification of DNA Fragments from Agarose Gel

In the following table the purification procedure for DNA from agarose gel slice is described:

1. Isolate DNA band of interest electrophoretically as follows.
• Load PCR reaction mixture on a 0.8 - 2% agarose gel.
• Use 1 × TAE or 1 × TBE as running buffer.
• Electrophorese until DNA band of interest is isolated from adjacent contaminating fragments.

2. Cut desired DNA band from gel using an ethanol-cleaned scalpel.

3. Determine gel mass by first pre-weighting the tube, and then reweighting the tube with the excised gel slice.

4. Add 300 µl **DR Buffer** (**Black Cap**) for every 100 mg agarose gel slice to the microcentrifuge tube.

5. Incubate the suspension for 5-10 min at 55°C. Vortex the tube briefly every 2 - 3 min during incubation.

6. After the agarose gel slice is completely dissolved, transfer the solution into the DNA spin column.

7. Centrifuge 30 - 60 s at 8000 g at room temperature.

8. Disconnect the Filter Tube, and discard the flowthrough solution. Reconnect the Filter Tube to the same Collection Tube.

9. Add 500 µl **Wash Buffer** (**Blue Cap**) to DNA spin column.

10. Centrifuge 1 min at maximum speed (8000 – 10000 g). Discard the flowthrough solution.

11. Centrifuge the DNA spin column at 10.000 g for 1 min to remove residual ethanol.

12. Reconnect the Filter Tube to a clean 1.5 ml microcentrifuge tube.

13. Add 50 - 100 µl **Elution Buffer** (**White Cap**) or sterile DDW directly onto column membrane and stand for 3 min.

14. Centrifuge at 10.000 g for 1 min. Store DNA at 4˚C or -20˚C.

Purification of PCR Products in Solution after Amplification

In the following protocol the purification of PCR products in solution after amplification is described.

1. Add 300 µl **DR Buffer** (**Black Cap**) for every 100 µl PCR product. Mix sample (Binding Buffer + PCR solution) well.

2. Transfer the sample from step 1 to DNA spin column.

3. Centrifuge 30 - 60 s at 8000 g at room temperature.

4. Disconnect the Filter Tube, and discard the flowthrough solution. Reconnect the Filter Tube to the same Collection Tube.

5. Add 500 µl **Wash Buffer** (**Blue Cap**) to DNA spin column.

6. Centrifuge 1 min at maximum speed (8000 – 10000 g). Discard the flowthrough solution.

7. Centrifuge the DNA spin column at 10.000 g for 1 min to remove residual ethanol.

8. Reconnect the Filter Tube to a clean 1.5 ml microcentrifuge tube.

9. Add 50 - 100 µl **Elution Buffer** (**White Cap**) or sterile DDW directly onto column membrane and stand for 3 min.

10. Centrifuge at 10.000 g for 1 min. Store DNA at 4˚C or -20˚C.

- Either use the eluted DNA directly or store the eluted DNA at +2 to +8°C or -15 to -25°C for later analysis.

Related DNA Extraction Products

Product	Package Size	Catalogue Number
Plasmid DNA Isolation Kit	25 preps	DV01025
Plasmid DNA Isolation Kit	50 preps	DV01050
Plasmid DNA Isolation Kit	100 preps	DV01100
DNA Gel Recovery Kit (PCR Purification Kit)	25 prep	DR02025
DNA Gel Recovery Kit (PCR Purification Kit)	50 preps	DR02050
Genomic DNA Extraction Kit from Gram Negative Bacteria	25 prep	DM04025
Genomic DNA Extraction Kit from Gram Negative Bacteria	50 prep	DM04050
Genomic DNA Extraction Kit from Gram Positive Bacteria	25 prep	DM05025
Genomic DNA Extraction Kit from Gram Positive Bacteria	50 prep	DM05050
Genomic Yeast DNA Extraction Kit	25 prep	DY06025
Genomic Yeast DNA Extraction Kit	50 prep	DY06050
Plant Total DNA Extraction Kit	50 prep	DP07050
Plant Total DNA Extraction Kit	100 prep	DP07100
Genomic DNA Extraction Kit from Seed	50 prep	DS08050
Genomic Fungi DNA Extraction Kit	25 prep	DF09025
Genomic Fungi DNA Extraction Kit	50 prep	DF09050
Blood DNA Extraction Kit	25 prep	DB10025
Blood DNA Extraction Kit	50 prep	DB10050
Blood DNA Extraction Kit	100 prep	DB10100
Tissue & Cells DNA Isolation Kit	50 prep	DT11050

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