# Gel DNA Recovery Kit

**For Research Use Only**

**Cat. No: EX6151** **Storage: RT**

**Shipment: RT** **Quantity: 25Reactions**

## KIT CONTENTS

| | |
|---|---|
| **Binding Buffer** | **30ml** |
| **Washing Buffer *** | **4ml** |
| **Elution Buffer **** | **2×1250 µl** |
| **Mini column** | **25** |
| **Protocol Handbook** | **1** |

*** Before first use, add absolute ethanol (ACS grade or better) into Washing Buffer (Add 16ml absolute ethanol in Washing Buffer before use)**

**** 10 mM Tris, pH 8.5**

## PRODUCT SPECIFICATIONS

| | |
|---|---|
| **Standard sample size** | **200 mg of gel (400 mg max.)** |
| **Recovered DNA size** | **80 bp~10 kb** |
| **Typical yields** | **~80%** |
| **Preparation time** | **~30 min** |
| **Minimum elution volume** | **30µl** |

## STORAGE CONDITIONS

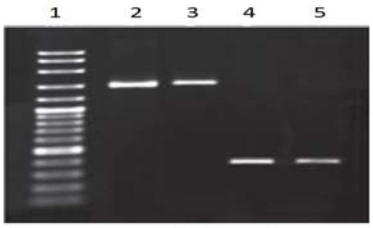
**All components should be stored at room temperature (15~25˚C). During shipment or storage under cool ambient condition, a precipitate can form in Binding buffer. In such a case, heat the bottle to 50˚C to dissolve completely. Using precipitated buffers will lead to poor DNA recovery. Gel DNA Recovery Kit is guaranteed until the expiration date printed on the product box.**

## SAFETY INFORMATION

**Binding buffer contains chaotropic salt, which is irritant. Take appropriate laboratory safety measuresandweargloveswhenhandling. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.**

## Introduction

**Gel DNA Recovery Kit provides reliable and fast method for a fast and efficient isolation of 80 bp to 10 kb of DNA fragments from standard or low-melting agarose gel in TAE or TBE buffer system. Purified DNA can be directly used in ligation, labelling, sequencing and many other downstream applications without further manipulation.**




1. DNA Ladder
2. DNA fragment 1.5 Kb
3. Recovered DNA fragment 1.5 Kb
4. DNA fragment 300bp
5. Recovered DNA fragment 300 bp

**General Considerations**

**Gel DNA Recovery Kit takes advantage of silica membrane and spin column technology to recover DNA fragments. Under high salt conditions, DNA binds to silica membrane and impurities pass through membrane into a collection tube. The membranes are washed with an ethanol-containing buffer to remove any traces of proteins, salts, remnants, of agarose and other enzymatic reaction components. Finally pure DNA is released into a clean collection tube with water or low ionic strength buffer.**

**pH Indicator**

**Binding and solubilization Binding buffer make the optimal binding condition in each specific applications. Binding buffer is composed to dissolve standard agarose gel as well as low melt agarose gel in addition to adjusting a binding condition. Usually low melt agarose gel results in a better recovery yield. DNA binds to silica membrane at lower pH than pH 7.5. The components and pH of starting sample can alter the pH of the mixture with binding buffer, especially in extraction of DNA from agarose gel. Binding buffer contains pH indicator in order to check this alteration of binding condition. If the color (around yellow) of binding mixture turns to red after addition of Binding buffer, it means that the pH of binding mixtures is higher than the optimal, and it can be easily adjusted with small volume of 3M sodium acetate,pH5.0, before proceeding with the protocol. The indicator dye is completely removed during subsequent washing step and does not interfere the downstream applications.**

**Washing**

**Any unwanted impurities, such as salts, proteins, nucleotides, agarose, dyes and detergents will not be bound but be passed through the silica membrane. A minute impurities, such as salts, are quantitatively washed away with Washing Buffer which contains ethanol. The quality of DNA can be slightly increased with the repeat of washing. Any residual ethanol should be removed completely with an additional centrifugation because the residual ethanol in eluate may interfere some subsequent applications.**

**Elution**

**DNA is released under the condition of low salts and neutral or weakly alkaline pH (7.0<pH<9.5). Although Buffer EB (10 mM Tris, pH 8.5), TE, or distilled water can be used for elution, it should be considered that EDTA in buffer TE may interfere the subsequent reactions and low pH (<7.0) of distilled water can reduce DNA recovery. Because water does not have any buffering agents the eluate in water should be stored under -20˚C not to degrade.**

**The minimum elution volume is 30 µl and lower volume will decrease the yield significantly. It is important for optimal elution to apply the elution buffer to the center of the membrane, because the membrane should be covered completely by eluent for an optimal recovery. Up to 200 µl of Elution Buffer can be applied and it results in low concentration of DNA. Higher concentrated DNA will be obtained with lower elution volume, and maximum yield can be obtained by larger elution volume. The yield with large fragments (>5 kb) can be increased slightly by using pre-warmed (70˚C) elution buffer. Incubation for 5 minute after addition of eluent may increase the efficiency of elution.**

**Additional equipment or materials to be supplied by the user**

- **Microcentrifuge**
- **Sterile 1.5 ml microcentrifuge tubes**
- **Water bath or heating block; 55°C**
- **Absolute ethanol**
- **Isopropanol**

**Protocol**

**Before experiment**

***Before first use, add absolute ethanol (ACS grade or better) to Washing Buffer as indicated on the bottle. (Add 16ml absolute ethanol in Washing Buffer before use)**

*** All centrifugation should be carried out at 10,000 x g above (>12,000 rpm) at room temperature in a microcentrifuge.**

*** Prepare water bath or heating block to 55˚C.**

*** All solutions should be equilibrated at room temperature before procedures.**

*** For large fragments (>5 kb), pre-warm Elution Buffer to 70˚C.**

***If a precipitate is formed in Binding buffer, heat to 50˚C to dissolve before use.**

1. **Excise the DNA band of interest using an ethanol-cleaned razor blade or scalpel on a transilluminator.**

**Minimize gel volume by cutting the gel slice as small as possible.**

**Use of long wave length transilluminator and short handling time will lead to better quality of DNA. It can be critical in some experiments, such as ligation.**

**2. Weigh the gel slice in a microcentrifuge tube. Add 3 volumes (µl) of Binding buffer to 1 volume (mg) of gel.**

**For example, add 300µl of Binding buffer to each 100 mg of agarose gel slice. For**

**>1.5% agarose gel, add 5 volumes of Binding buffer.**

**3. Incubate at 55°C until the agarose gel is completely melted (5~10 min).**

**To help the efficient dissolving of gel, vortex the tube every 2~3 min during the incubation.**

**4. After the slice has dissolved completely, check that the color of the**

**mixture is yellow (similar to Binding buffer).**

**If the color of the mixture becomes red or purple, add 10 µl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow. Adjusting of pH is not needed when the change of color is due to the ingredient of loading dye (eg. bromophenol blue, xylene cyanol).**

**5. Add 1 gel volume of isopropanol to the sample and vortex to mix.**

**For 100 mg of gel volume, add 100 µl of isopropanol.**

**DO NOT centrifuge at this step.**

**6. Transfer the mixture to a Column. Centrifuge for 2 min at 10,000 x g above (>12,000 rpm). Discard the pass-through and re-insert the mini column into the collection tube.**

**If the mixture volume is larger than 700 µl, apply the mixture twice; apply 700 µl of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat the step again until all of the mixture has been applied to the mini column.**

**7. Add 700 µl ofWashing Buffer to the mini column. Centrifuge for 2 min at 10,000 x g above (>12,000 rpm). Discard the pass-through and re-insert the mini column into the collection tube.**

**If the purified DNA will be used for salt sensitive applications, let the mini column stand for 5 min after addition of Washing Buffer, making some amount of wash buffer flow through the column by gravity before centrifugation.**

**8. Centrifuge for an additional 2 min at full speed to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube.**

**If residual ethanol remains in the mini column, centrifuge again for an additional 1 min at full speed before transferring to a new 1.5 ml microcentrifuge tube. Residual ethanol from Wash Buffer can inhibit subsequent enzymatic reaction.**

**9.Apply 30-50µl of Elution Buffer or ddH2O to the center of the membrane in the mini column, let stand for 5-10 min in 55 ˚Cand centrifuge for 2min at 10,000 x g above (>12,000 rpm).**

**10.Repeat step 9.**

**For higher overall yield, increase the volume of Elution Buffer and repeat the elution step once again. Optimal results may be obtained by eluting twice.**

**More 20~40% DNA can be acquired by repeat of eluting once again. A new 1.5ml tube can be used to prevent dilution of the first eluate.**

**For long-term storage, eluting in Elution Buffer (10 mM Tris, pH 8.5) or TE, pH8.0 and storing under -20˚C is recommended. When using water for elution, check the pH of water (>pH 7.0) before elution.**

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## Troubleshooting Guide

| Facts | Possible Causes | Suggestions |
|---|---|---|
| Low or no recovery | Incompletly solubilized gel | The sliced agarose gel should be completely dissolved without any particles. To assist the complete solubilization, mix the tube by vortexing every 2~3 minutes during incubation or increase the incubation time. To use low melt agarose usually results in better recovery. |
| | Too high pH of binding mixture | At high pH, the binding of DNA to silica membrane will be significantly reduced. The dye included in Binding buffer indicates the pH of mixture as color change from yellow at optimal pH to brown or purple at abnormally higher pH. If the color of mixture has turned to brown or purple, add 10 µl of 3 M sodium acetate, (pH 5.0) to the sample and mix. The color of mixture will turn to yellow indicating the correct pH for DNA binding. |
| | Improper Elution Buffer | As user's requirement, elution buffer other than Elution Buffer can be used. The condition of optimal elution is low salt concentration with alkaline pH (7.0<pH<9.5). When water or other buffer was used as eluent, ensure that conditions. |
| | Elution Buffer incorrectly dispensed | Ensure that Elution Buffer dispensed to the center of membrane. Incorrectly dispensed Elution Buffer causes inappropriate contact with membrane, followed by poor DNA recovery. |
| Ligation failure | Too long or strong exposure to UV on transilluminator | UV destroys the DNA ends. Use UV of long wave length and make the handling time as short as possible when excising the gel slice. |
| Clogged membrane | Incompletly solubilized gel | See the section 'Incompletely solubilized gel' in the Facts "Low or no recovery" |
| | >1.5% agarose gel is used | For >1.5% agarose gel, 5 volumes of Binding buffer to 1 volume of gel slice should be added. For 100 mg of agarose gel, add 500 µl of Binding buffer. If the mini column is clogged, transfer the mixture from the mini column to a 1.5 ml microcentrifuge tube, add 1 volume of Binding buffer to mixture volume. Incubate for 5 minutes at 55˚C, proceed again to binding steps. |
| Enzymatic reaction is not performed well with the purified DNA | Residual ethanol from Wash Buffer remains in eluate | It is essential to remove any residual ethanol included in Wash Buffer from column membrane. Centrifuge again for complete removal of ethanol. |
| | Too high salt concentration in eluate | Incubate for 5 minutes after addition of Washing Buffer at washing steps. |
| | Eluate contains denatured ssDNA | For reannealing of ssDNA to dsDNA, incubate ssDNA at 95˚C for 2 minutes, and then allow to cool slowly to room temperature. |
| DNA floats out while loading on agarose gel | Residual ethanol from Washing Buffer remains in eluate | It is essential to remove any residual ethanol included in Washing Buffer from column membrane. Centrifuge again for complete removal of ethanol. |
| Non-specific band appears after purification | DNA denatured | Renature the DNA by warming up to 95˚C for 1 minute and let cool slowly to room temperature |