

## Gel-PCR 2in1 Kit- HiPure DNA Kit

### Introduction

Gel-PCR 2in1 Kit-HiPure DNA Kit uses the HiPure technology to recover DNA Fragments between 60bp-10kbp with yields exceeding 80%. DNA is suitable for ligations, PCR,sequencing,restriction digestion, orvarious labeling reactions. In addition,this kit can be also used to recover DNA directly from crude genomic DNA, enzymatic reactions such as PCR, enzyme digestion reactions.

### Principle

The HiPure system uses a simple bind-wash-elute procedure. Gel slices are dissolved in a buffer containing a pH indicator, allowing easy determination of the optimal pH for DNA binding, and the mixture is applied to the column. Nucleic acids adsorb to the silica-gel membrane in the high-salt conditions provided by the buffer. Impurities are washed away and pure DNA is eluted with a small volume of low-salt buffer provided or water, ready to use in subsequent applications.

The kit can be used to purify PCR products or DNA from other enzymatic reactions.

### Kit Contents

Product	D211101	D211103
Number of Preps	50 Preps	250 Preps
Buffer GDP	50 ml	250 ml
Buffer DW2*	20 ml	2 x 50 ml
Elution Buffer	6 ml	30 ml
HiPure DNA Mini Columns II	50	250
2 ml Collection Tubes	50	250

### Storage and stability

The Kit can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions. If any precipitates form in the buffers, warm at 37°C to dissolve.

## Binding Capacity

HiPure DNA Mini column II can bind ~20µg DNA

## Materials and Equipment to be Supplied by User

- Add 80ml (50 Preps) or 2 x 200ml (250 Preps) 100% ethanol to the bottle of Buffer DW2 and store at room temperature.
- Heat block or water bath capable of 50~55°C

## Protocol 1: Pure DNA from agarose gel

1. Excise the DNA fragment from the agarose gel with a clean&sharp scalpel. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5ml microcentrifuge tube.
2. **Add 2 volume of Buffer GDP to 1 volume of the gel (100 mg gel approximately 100µl ). Incubate at 50~55°C for 10 min or until the gel has completely melted.** Vortex or shake the tube every 2-3 min during the incubation.
3. Insert a HiPure DNA Mini Column II in a 2ml Collection Tube.
4. **Add no more than 750µl DNA/agarose solution from step 2 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
5. Repeat Step 4 until all of the sample has been transferred to the column.
6. **Add 300µl Buffer GDP to the column and incubate for 1 min.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
7. **Add 650µl Buffer DW2 to the column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
8. **Add 650µl Buffer DW2 to the column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
9. Centrifuge the empty Column at 12,000 × g for 2 minute at room temperature to dry the column matrix.
10. Transfer the Column to a clean 1.5ml microcentrifuge tube. **Add 25~50 µl Elution Buffer or deionized water directly to the center of the column membrane.** Let it sit at room temperature

for 2 minutes.

When DNA are >5 kb, add 25~50µl preheat Elution Buffer (or water) to 70°C to the membrane and incubate for 5min. For maximal recovery, repeat step 10 once by eluate.

A second elution step with a further 25~50µl Elution Buffer will increase yields by up to 15%. Elution with volumes of less than 25µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

11. Centrifuge at 12,000 × g for 1 minute at room temperature. Store DNA at -20°C.

## Protocol 2 : Pure DNA from PCR Products or Enzyme Reactions

1. Determine the volume of your sample. Transfer the sample into a clean 1.5ml microcentrifuge tube.
2. **Add 2 volumes Buffer GDP to the sample and mix well.**
  - For PCR Products smaller than 80bp: Add 1 Volumes Buffer GDP and 2 volumes of absolute ethanol to the sample, mix well.
  - For gDNA product(>2ug): Add 1 Volumes Buffer GDP and 1 volumes of absolute ethanol to the sample, but If gDNA Products contains a lot of pigments and impurities, add 2 volume of Buffer GDP to the sample, mix well.
  - For gDNA product(<2ug): Add 2 volume of Buffer GDP to the sample and mix well.
3. Insert a HiPure DNA Mini Column II in a 2ml Collection Tube.
4. **Add no more than 700µl of the Mixture from Step 2 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
5. Repeat Step 4 until all of the sample has been transferred to the column.
6. **Add 450µl Buffer DW2 to the column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
7. Repeat Steps 6 for a second Buffer DW2 wash step.
8. Centrifuge the empty Column at 12,000 × g for 2 minute at room temperature to dry the column matrix.
9. Transfer the Column to a clean 1.5ml microcentrifuge tube. **Add 25-50µl Elution Buffer or**

deionized water directly to the center of the column membrane. Let sit at room temperature for 2 minutes. Centrifuge at  $12,000 \times g$  for 1 minute and store DNA at  $-20^{\circ}\text{C}$ .

When DNA are  $>5$  kb, add  $30\sim 50\mu\text{l}$  preheat Elution Buffer (or water) to  $70^{\circ}\text{C}$  to the membrane and incubate for 5min. For maximal recovery, repeat step 10 once by eluate.

A second elution step with a further  $25\sim 50\mu\text{l}$  Elution Buffer will increase yields by up to 15%. Elution with volumes of less than  $25\mu\text{l}$  increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield).

## Troubleshooting Guide

### 1. Low or no recovery

- **Buffer DW2 did not contain ethanol:** Ethanol must be added to Buffer DW2 before used. Repeat procedure with correctly prepared Buffer DW2.
- **Inappropriate Elution Buffer:** DNA will only be eluted efficiently in the presence of low salt buffer or Water.
- **Gel slice incompletely solubilized:** After Addition of Buffer GDP to the gel slice, mix by vortexing the tube every 2-3 minutes during the  $50\sim 55^{\circ}\text{C}$  incubation.
- **Sample volume too high or low:** for reaction cleanup, The sample volume must be in the range of  $20\sim 200\mu\text{l}$ .

### 2. DNA does not perform well (e.g. in ligation reaction)

- **Salt concentration in eluate too high:** Modify the wash step by incubating the column for 5 min at room temperature after adding  $650\mu\text{l}$  of Buffer DW2, then centrifuge.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at  $>12,000 \times g$  for 1 min.
- **Eluate contaminated with agarose:** The gel slice is incompletely solubilized or weighs  $>400\text{mg}$ . Be sure to vortex the gel slice in Buffer GDP every 2-3 minutes during the solubilization step.



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