HiPure Gel DNA Micro Kit

Introduction

HiPure Gel DNA Micro Kit uses proprietary chemistry and 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.

Kit Contents

Product	D211002	D211003
Purification times	100 Preps	250 Preps
Buffer GDP	60 ml	125 ml
Buffer DW1	40 ml	90 ml
Buffer DW2*	20 ml	50 ml
Elution Buffer	20 ml	30 ml
HiPure DNA Micro Columns	100	250
2 ml Collection Tubes	100	250

Storage and stability

The Kit should 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 Micro column can bind ~4μg DNA.

Materials and Equipment to be Supplied by User

- Dilute Buffer DW2 with 80ml (100 Preps) or 200ml (250 Preps) 100% ethanol and store at room temperature。
- Heat block or water bath capable of 50~55℃

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℃ for 7 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 Micro Column in a 2ml Collection Tube.
4. **Add no more than 650μl DNA/agarose solution from step 2 to the Column.** Centrifuge at 10,000 × 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 DW1 to the column. Incubate at room temperature for 1 min.** Centrifuge at 10,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.
7. **Add 450μl Buffer DW2 to the column.** Centrifuge at 10,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
8. **Add 450μl Buffer DW2 to the column.** Centrifuge at 10,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
9. Centrifuge the empty Column at 10,000 × g for 2 minute at room temperature to dry the column matrix.
10. Transfer the Column to a clean 1.5ml microcentrifuge tube. **Add 10~50 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 Eluiton Buffer (or water) to 70°C to the membrance and incubate for 5min. For maximal recovery, repeat step 10 once by eluate. A second elution step with a further 10~50μl Elution Buffer will increase yields by up to 15%. Elution with volumes of less than 10μl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield).

11. Centrifuge at 10,000 × g for 1 minute at room temperature. Store DNA at -20℃.

Protocol 2 : Pure DNA from PCR Product or Enzyme Reaction

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 Micro Column 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 10-50μl 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 × g for 1 minute and store DNA at -20℃.
When DNA are >5 kb, add 25~50μl preheat Eluiton Buffer (or water) to 70°C to the membrance and incubate for 5min. For maximal recovery, repeat step 10 once by eluate.
A second elution step with a further 10~50μl Elution Buffer will increase yields by up to 15%.
Elution with volumes of less than 10μl 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 precedweure with correctly prepare Buffer PE.
- **Inappropriate Elution Buffer:** DNA will only be eluted efficiently in the presende 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 50~55°C incubation.
- **Sample volume too high or low:** for reaction cleanup, The sample volume must be in the range of 20~200ul.

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 650μl of Buffer DW2, then centriufge.
- **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 x g for 1min.
- **Eluate contaminated with agarose:** The gel slice is incompletely solubilized or weighs >400mg. Be sure to vortex the gel slice in Buffer GDP every 2-3 minutes during the solubilization step.