HiPure Clean Up 96 Kit

Introduction

HiPure DNA Clean Up 96 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 enzymatic reactions such as PCR and 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	D212201	D212202	D211203
Purification times	1 x 96 Preps	4 x 96 Preps	20 x 96 Preps
Buffer GDP	120 ml	400 ml	ml
Buffer DW2*	20 ml	100 ml	3 x 100 ml
Elution Buffer	20 ml	20 ml	30 ml
HiPure DNA Plate	1	4	20
2.2ml Collection Plate	1	4	20
1.6 ml Collection Plate	1	4	20
0.8ml Collection Plate	1	4	20

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℃ to dissolve.

Binding Capacity

HiPure DNA Plate can bind ~20μg DNA

Materials and Equipment to be Supplied by User

- Dilute Buffer DW2 with 80ml (1x90 Preps), 400ml (4x96 Preps) or 3 x 400ml (20 x 96 Preps) 100% ethanol and store at room temperature。
- Heat block or water bath capable of 50~55℃

Protocol

- Agorase 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 2.2ml Collection Plate.
2. **Add 2~3 volume of Buffer GDP to 1 volume of the gel (100 mg gel approximately 100μl). Incubate at 50~55℃ for 10~15 min or until the gel has completely melted.** Shake the plate every 2-3 min during the incubation.

- PCR Product or Enzyme Reactions

1. Determine the volumes of your samples. Transfer the samples into a clean 2.2ml Collection Plate.
2. **Add 2 volumes Buffer GDP to the sample and mix well.**

- For DNA Size less than 80bp: Add 1 Volume of Buffer GDP and 2 volumes of absolute ethanol to the sample, mix well.
- For gDNA products: Add 1 Volumes Buffer GDP and 1 volumes of absolute ethanol to the sample and mix well.
- For gDNA products(Pigments): If gDNA Products contains pigments and impurities, add 2 volume of Buffer GDP to the sample, mix well.

Spin Procedure

3. Place a new HiPure DNA Plate in a 1.6ml Collection Tube.

4. **Add no more than 750µl of the sample from step 2 to the Plate.** Centrifuge at 4,000 × g for 3 minute at room temperature. Discard the filtrate and reuse collection Plate.

5. Repeat Step 4 until all of the sample has been transferred to the Plate.

6. **(Only for agarose Gel) Add 300µl Buffer GDP to the Plate.** Incubate for 1 min and centrifuge at 4,000 × g for 3 minute at room temperature. Discard the filtrate and reuse collection plate.

7. **Add 700µl Buffer DW2 to the Plate.** Centrifuge at 4,000 × g for 3 minute at room temperature. Discard the filtrate and reuse collection plate.

8. **Add 700µl absolute ethanol to the Plate.** Centrifuge at 4,000 × g for 3 minute at room temperature. Discard the filtrate and reuse collection plate.

9. Centrifuge the empty Plate at 4,000 × g for 10 min at room temperature to dry the plate matrix. Incubate the plate at room temperature for another 15 min to dry the matrix.

10. Transfer the HiPure DNA Plate into a new 0.8ml Collection Plate. Add 75~100µl Elution Buffer (10mM Tris·Cl, pH 8.5)to the center of the membrane. let the column stand for 2 min.Centrifuge at 4,000 × g for 3 minute at room temperature.

    Important: Ensure that the elution buffer is dispensed directly onto the center of HiPure membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

Vacuum Procedure:

3. Plate a new HiPure DNA Plate into Vacuum Manifold such as QiaVac 96.

4. load the samples from step 2 into the plate by pipetting,and then apply vacuum. After the samples have passed through the column, switch off the vacuum source. The maximum loading volume of the column is 800 µl. For sample volumes greater than 1000 µl, simply load again.

5. **(Only for agarose gel) To wash, add 0.3ml of Buffer GDP and incubate for 1 min. Apply vacuum.**

6. **To wash, add 0.75 ml of Buffer DW2 to each column and apply vacuum.**

7. **To wash, add 0.75 ml of absoluet ethanol to each column and apply vacuum.** After Absoluet ethanol in all wells has passed through the membrane, apply maximum vacuum for an additional 10 min to dry the membrane.

    Important: This step removes residual ethanol from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator valves or leakage valves if they

are used), allowing maximum airflow to go through the wells.

8. Switch off vacuum source. Vigorously tap the HiPure DNA plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the plate with clean absorbent paper.

   This step removes residual Absoluet ethanol from around the outlet nozzles and collars of the k plate. Residual ethanol may inhibit subsequent enzymatic reactions, e.g., sequencing.

9. Transfer the HiPure DNA Plate into a new 0.8ml Collection Plate. Add 75~100μl Elution Buffer (10 00mM Tris·Cl, pH 8.5)to the center of the membrane. let the column stand for 2 min. Centrifuge at 4,000 × g for 3 minute at room temperature.

   Important: Ensure that the elution buffer is dispensed directly onto the center of HiPure membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 μl from 80 μl elution buffer volume, and 40 μl from 60 μl elution buffer volume.

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.