HiPure DNA Clean Up Kit

Introduction

HiPure DNA Clean Up Kit uses proprietary chemistry and HiPure technology to recover DNA Fragments between 20bp-20kbp with yields exceeding 80%. DNA is suitable for ligations,PCR,sequening,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.

Kit Contents

Product	D214102	D214103
Purifications Times	100 Preps	250 Preps
Buffer GWP	30 ml	60 ml
Buffer DW2*	20 ml	50 ml
Elution Buffer	30 ml	60 ml
HiPure DNA Mini Columns I	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℃ to dissolve.

Materials and Equipment to be Supplied by User

- Add 80ml (100 Preps) or 200ml (250 Preps) 100% ethanol to the bottle of Buffer DW2 and store at room temperature。
- Microcentrifuge capable of at least 10,000 × g

Binding Capacity

HiPure DNA Mini column I can bind ~20ug DNA

Protocol

1. Briefly centrifuge the PCR Product/enzymatic reaction/genomic DNA tube to collect any drops from the inside of the lid.
2. Determine the volume of the reaction and transfer the sample into a clean 1.5ml microcentrifuge tube. Add Water or Buffer TE to total volume of 200µl.
3. **Add 200µl Buffer GWP to the tube and mix thoroughly by vortex for 5s.**
4. **Add 200µl(>100bp DNA) or 600µl (<100bp DNA) absolute ethanol to the sample.** Vortex to mix thoroughly.

Spin Procedure

5. Insert a HiPure DNA Mini Column I in a 2ml Collection Tube.
6. **Add no more than 700µl of the sample from step 4 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
7. Repeat Step 6 until all of the sample has been transferred to the column.
8. **Add 750µl 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. To elute DNA, add 20~50μl Elution Buffer to the center of the membrane. let the column stand for 2 min, and then centrifuge.

Vacuum Procedure:

5. Prepare the vacuum manifold following manual.

6. Insert up to HiPure DNA columns into the luer extensions of the Vacuum manifolds. Close unused positions with luer caps, and then connect manifolds to a vacuum source.

7. load the samples from step 4into the columns by decanting or 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 800 μl, simply load again.

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

9. Transfer each column to a 2ml collection tubes. Centrifuge for 1 min at 17,900 x g (13,000 rpm).

10. Place each column into a clean 1.5 ml microcentrifuge tube.

11. To elute DNA, add 20~50μl Elution Buffer to the center of the membrane. let the column stand for 2 min, and then centrifuge.

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.
- **Sample volume too high or low:** for reaction cleanup, The sample volume must be in the range of 20~200μl.

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.