HiPure PCR Pure Micro/Mini Kit

Introduction

HiPure PCR Pure Mini Kit uses proprietary chemistry and HiPure technology to recover DNA Fragments between 60bp-20kbp 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. Binding buffer is added directly to the PCR sample or other enzymatic reaction, and the mixture is applied to the column.Nucleic acids adsorb to the silica 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 all subsequent applications.

Kit Contents

Product	HiPure PCR Pure Micro Kit		HiPure PCR Pure Mini Kit	
	D212002	D212003	D212102	D212103
Purification times	100 Preps	250 Preps	100 Preps	250 Preps
Buffer DP	60 ml	120 ml	60 ml	120 ml
Buffer DW2*	20 ml	50 ml	20 ml	50 ml
Elution Buffer	15 ml	30 ml	15 ml	30 ml
HiPure DNA Micro Columns	100	250	-	-
HiPure DNA Mini Columns II	-	-	100	250
2 ml Collection Tubes	100	250	100	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 Micro Column can bind 10ug DNA. HiPure DNA Mini column II can bind 35ug DNA.

Materials and Equipment to be Supplied by User

- Add 80ml (100 Preps) or 200ml (250 Preps) 100% ethanol to the bottle of DW2 and store at room temperature.

Spin Procedure

1. **Add 5 volumes of Buffer DP to 1 volume of the PCR sample and mix well.** It is not necessary to remove mineral oil or kerosene.

   For example, add 500µl of Buffer DP to 100µl PCR sample (not including oil).

2. Insert a HiPure DNA Column in a 2ml Collection Tube.
3. **Add no more than 650µl of the sample from step 1 to the Column.** Centrifuge at 10,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
4. Repeat Step 3 until all of the sample has been transferred to the column.
5. **Add 700µl Buffer DW2 to the column.** Centrifuge at 10,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
6. Centrifuge the empty Column at 12,000 × g for 2 minute at room temperature to dry the column matrix.
7. To elute DNA, add 10~50µl Elution Buffer (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the membrane. let the column stand for 1 min, and then centrifuge.

   HiPure DNA Micro Column, add 10~30µl Elution Buffer. Hiure DNA Mini Column I, add 30~50µl Elution Buffer. A second elution step with a further Elution Buffer will increase yields by up to 15%.

Vacuum Procedure:

1. **Add 5 volumes of Buffer DP to 1 volume of the PCR sample, and then mix.** It is not necessary to remove mineral oil or kerosene.

   For example, add 500 µl of Buffer DP to 100 µl PCR sample (not including oil).

2. Prepare the vacuum manifold following manual.

3. 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.

4. **load the samples from step 1into 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.

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

6. To wash, add 0.5ml of Absolute ethanol to each column and apply vacuum.

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

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

8. To elute DNA, add 10~50µl Elution Buffer or water to the center of the membrane. let the column stand for 1 min, and then centrifuge. HiPure DNA Micro Column, add 10~30µl. Hiure DNA Mini Column I, add 30~50µl.

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 DW2.
- **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 20~200ul.

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 650µlof 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.