【Product Name】HiPure DNA Micro Kit

【Product specifications】50 Preps/Kit, 250 Preps/Kit

【Intended Use】

This product provide fast and easy methods for purification of total DNA for reliable PCR and Southern blotting. Total DNA(e.g., genomic, viral, mitochondrial) can be purified from small volume of blood, tissue and dry blood spots.

【Principle】

This product is based on silica column purification. The sample is lysed and digested with lysate and protease, DNA is released into the lysate. Transfer to an adsorption plate and filter column. Nucleic acid is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, Nucleic acid was finally eluted with low-salt buffer.

【Kit Contents】

Cat.No.	D312502	D312503	Main Composition
Purification Times	50	250	-
HiPure DNA Mini Columns I	50	2 x 125	Silicon Column
2ml Collection Tubes	100	5 x 100	PP Column
Buffer ATL	15 ml	60 ml	Tris/EDTA/SDS
Buffer AL	15 ml	60 ml	Tween-20/Guanidine Salt
Buffer GW1*	22 ml	66 ml	Guanidine Salt
Buffer GW2*	20 ml	2 x 50 ml	Tris/NaCl
Carrier RNA	310 μg	2 x 310 μg	Poly A
Proteinase K	24 mg	120 mg	Proteinase K
Protease Dissolve Buffer	1.8 ml	10 ml	Glycerol/Tris/CaCl2
Buffer AE	15 ml	60 ml	Tris/EDTA

【Storage conditions and Validity】

Carrier RNA and Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15–25°C) does not affect its performance. The remaining kit components can be stored dry at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

【Preparation before Use】

- Add 80ml (50Preps) or 200ml (250Preps) absolute ethanol to each bottle of Buffer GW2 and store at room temperature.
- Add 28ml (50Preps) or 84ml (250Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 1.2ml (50Preps) or 6ml (250Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Add 0.31ml Bufer AE to each tube of Carrier RNA (310μg) and store at -20℃ after dissolve.
- Ethanol (96 – 100%)

Carrier RNA

The kit is supplied with carrier RNA, which can be added to Buffer AL if required. Carrier RNA enhances binding of DNA to the HiPure column membrane, especially if there are very few target molecules in the sample.For purification of DNA from very small amounts of sample, such as low volumes of blood (10ul), we recommend adding 1~2ug carrier RNA to Buffer AL. For samples containing larger amounts of DNA, addition of carrier RNA is optional. If carrier RNA is used, eluates from columns contain both sample DNA and carrier RNA, with the amount of carrier RNA greatly exceeding the amount of DNA.

Protocol

A. Small Volumes of Blood

1. Pipet 1–100 μl whole blood into a 1.5 ml microcentrifuge tube (not provided) and add Buffer ATL to a final volume of 100 μl.
2. Add 10μl proteinase K and 100μl Buffer AL, close the lid, and mix by pulse-vortexing for 15 s.
   To ensure efficient lysis, it is essential that the sample, Buffer ATL, proteinase K, and Buffer AL are thoroughly mixed to yield a homogeneous solution. If the volume of blood is lower than 10μl, we recommend adding 1~2μl carrier RNA to Buffer AL.
3. Incubate at 56°C for 10 min.
4. Add 100μl absolute ethanol to the samples, vortex at maximum speed for 10 seconds.

B. Blood stains/Seminal Spots

1. Transfer the 1~3 slices(3mm) to the 2.0ml centrifuge tube. **Add 200μl Buffer ATL and 20μl Proteinase**

K to the sample. Shaking at 900-1200rpm for 60min at 55°C.

2. **Add 200µl Buffer AL to the samples, Shaking at 900-1200rpm for 10 min at 70°C.**

   Note: If processing only 1 blood card punch with a diameter of 3 mm or less, we recommend adding 1µl carrier RNA to Buffer AL. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

3. Centrifuge at 13,000 x g for 1 min.

4. **Transfer the supernatant to a new centrifuge tube, add 200µl absolute ethanol, then vortex to mix.**

C. Urine

1. **Transfer up to 1 ml urine to a 1.5 ml microcentrifuge tube (not provided) and centrifuge at 6000 x g (8000 rpm) for 2 min.**

   If the volume of the urine sample is between 1 and 10 ml, pellet the cells in an appropriately sized centrifugation tube.

2. **Discard the supernatant and add 500 µl Buffer AE. Vortex for 5 s and c**entrifuge at 6000 x g (8000 rpm) for 2 min.

3. Discard the supernatant, add 200µl Buffer ATL and 20µl proteinase K to the pellet. Incubate at 56°C, with shaking at 900 rpm, for 1 h.

   Adding 10 µl 1 M DTT may increase sensitivity, since urine can contain sperm cells which can only be lysed in the presence of reducing agents such as DTT or β-mercaptoethanol.

4. **Add 200µl Buffer AL and 200µl ethanol (96–100%) and mix by pulse-vortexing for 10 s.**

   Note: We recommend adding 1ul carrier RNA to Buffer AL. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL

D. solid tissue

1. Cut <10 mg tissue into small pieces and transfer into a new 1.5ml centrifuge tube.

2. Add 200µl Buffer ATL and 20µl Proteinase K, shake at 55°C for 30~120 minutes or overnight until completely digested.

3. Add 200µl Buffer AL to the samples and vortex for 15 seconds. Incubation at 70°C for 10 minutes.

4. Add 200µl absolute ethanol to the samples, vortex at maximum speed for 10 seconds.

Column purification

5. Insert a HiPure DNA Mini Column I in a 2ml Collection Tube.

6. **Add the mixture from Step 4 to the Column.** Centrifuge at 10,000 × g for 1 minute at room temperature. Discard the filtrate and collection tube.

7. **Insert the column in a new 2ml Collection Tube. Add 500µl Buffer GW1to the column.** Centrifuge at 10,000 × g for 1 minute.Discard the filtrate and reuse collection tube.

8. **Add 500µl Buffer GW2 to the column.** Centrifuge at 10,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.

9. **Add 500µl Buffer GW2 to the column.** Centrifuge at 10,000 × g for 1 minute at room temperature.Discard the filtrate and reuse collection tube.

4. Centrifuge the empty column at 10,000 × g for 2 minute at room temperature to dry the column matrix. Open the lid and incubate at room temperature for 10 min to dry the matrix.

5. **Place the column to a clean 1.5ml microcentrifuge tube. Add 30~100µl Buffer AE directly to the center of the column membrane.** Let sit at room temperature for 3-5 minutes.

6. Centrifuge at 12,000 × g for 1 minute at room temperature. Store DNA at -20℃.

   A second elution step with a further 30~100µl Buffer AE will increase yields by up to 15%. Elution with volumes of less than 30µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield).

Troubleshooting Guide

1. Low or no recovery

- **Buffer GW1/GW2 did not contain ethanol:** Ethanol must be added to Buffer GW1/GW2 before used. Repeat procedure with correctly prepare Buffer.
- **Low concentration of target DNA in the Sample:** Samples were standing at room temperature for too long. Repeated freezing and thawing should be avoided.

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 Buffer GW2, then centriufge or Vacuum.
- **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, then dry.
- **Inappropriate elution volume used:** Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.