【Product Name】HiPure Insect DNA 96 Kit

【Product specifications】96 Preps/Kit, 4 x 96 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 tissue and culture cells.

【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.	D313901	D313902
Purification Times	1 x 96 Preps	4 x 96 Preps
HiPure DNA Plate	1	4
2.2 ml Collection Plate	1	4
1.6 ml Collection Plate	1	4
0.5ml Collection Plate	1	4
Seal Film	8	32
Buffer ITL	30 ml	120 ml
Buffer IL*	30 ml	125 ml
Buffer GW1*	44 ml	2 x 110 ml
Buffer GW2*	50 ml	3 x 50 ml
Proteinase K	50 mg	200 mg
Protease Dissolve Buffer	6 ml	15 ml
Buffer AE	20 ml	60 ml

【Storage conditions and Validity】

Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 24 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 c onditions.The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved befor e use. Make sure that all buffers are at room temperature when used.

【Preparation before Use】

- Add 200ml absolute ethanol to each bottle of Buffer GW2 and store at room temperature.
- Add 56ml (96Preps) or 2 x 140ml (4 x 96Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 2.5ml (96Preps) or 10ml (4 x 96Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Add 30ml (96Preps) or 125ml (4 x 96Preps) absolute ethanol to the bottom of Buffer IL and store at room temperature.

【Protocol for tissue 】

1. Pipet 20 µl Proteinase K into the bottom of a 2.2ml Collection Plate.
2. Cut up to up to 10 mg of tissue into small pieces and place it in bottom of 2.2ml Collection Plate.
3. Add 250µl of Buffer ITL and seal the wells thoroughly using the Seal film. Incubate at 55°C until the tissue is completely lysed in an incubator oven.
4. Remove the seal film and add 500µl Buffer IL to the sample. Seal 96-well plate with an new seal film and mix again by shaking at 700~900rpm for 3 min.
5. Insert a HiPuregDNA Plate onto a 1.6mL Collection Plate (provided).
6. Pipet 750µl of the mixture from step 4 into HiPure DNA plate placed in a 1.6ml collection plate (supplied). Seal the DNA plate with an Seal film. Centrifuge for 5 min at >4,000 x g and discard the flow-through. Reuse collection plate.

7. **Remove the seal film and add 500µl Buffer GW1 to each well of plate.** Seal DNA Plate with seal film and centrifuge for 3 min at >4,000 x g . Discard the flow-through and reuse the collection plate.

8. **Remove the seal film and add 750µl Buffer GW2 to each well of the plate .** Seal HiPure DNA Plae with seal film and centrifuge for 3 min at >4,000 x g.

9. **Remove the seal film and add 750µl Buffer GW2 to each well of the plate .** Seal HiPure DNA Plae with seal film and centrifuge for 3 min at >4,000 x g.

10. Discard the flow through and reuse the collection plate. Centrifuge at >4,000 x g for 10 min.

11. Allow to air dry for 15-20 min at room temperature.

12. Transfer the plate onto a 0.5 ml Collection Plate, and pipet 75~100µl Buffer AE directly onto the membrane.

13. Incubate for 5 min at room temperature, and then centrifuge for 3 min at >4,000 x g to elute.

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.
- **Inefficient cell lysis due to insufficient mixing with Buffer ITL:** Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer ITL immediately and thoroughly by pulse-vortexing.
- **Low-percentage ethanol used instead of 100%:** Repeat the purification procedure with a new sample. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

2. A260/A280 ratio for purified nucleic acids is low

- **Inefficient cell lysis due to insufficient mixing with Buffer ITL:** Repeat the procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse vortexing.
- **Inefficient cell lysis due to decreased protease activity:** Repeat the DNA purification procedure with a new sample and with freshly prepared Proteinase K stock solution. Besure to store the stock solution at -20–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer ITL.
- **No ethanol added to the lysate before loading onto the column:** Repeat the purification procedure with a new sample.

3. 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 3 min at room temperature after adding 500ul of 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 >10,000 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.