【Product Name】HiPure Insect DNA Kit

【Product specifications】20 Preps/Kit, 50 Preps/Kit, 250 Preps/Kit

【Intended Use】

HiPure Insect DNA Kits provides a simple and rapid solution for total DNA extraction from insect tissue samples (<10 mg). This kit is based on silica gel column purification technology without toxic phenol chloroform extraction and time-consuming alcohol precipitation. The whole extraction process only takes 30 minutes. The purified DNA can be directly used in PCR, Southern blot, viral DNA detection, ect. .

【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.	D312901	D312902	D312902
Purification Times	20 Preps	50 Preps	250 Preps
HiPure DNA Mini Columns I	20	50	250
2 ml Collection Tubes	20	50	250
Buffer ITL	10 ml	30 ml	120 ml
Buffer IL*	10 ml	30 ml	120 ml
Buffer GW1*	13 ml	22 ml	110 ml
Buffer GW2*	10 ml	20 ml	2 x 50 ml
Proteinase K	12 mg	24 mg	120 mg
Protease Dissolve Buffer	1.8 ml	1.8 ml	15 ml
Buffer AE	10 ml	15 ml	60 ml

【Storage conditions and Validity】

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 their performance. The remaining kit components c an be stored at room temperature (15–25°C) and are stable for at least 18 months under these co nditions.

【Preparation before Use】

- Add 10ml (20 Preps), 30ml (50 Preps) or 120ml (250 Preps) absolute ethanol to the botthe of Buffer IL and store at room temperature.
- Add 17ml (20 Preps), 28ml (50 Preps) or 140ml (250 Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 40ml (20 Preps), 80ml (50 Preps) or 2 x 200ml (250 Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature
- Add 0.6ml (20 Preps), 1.2ml (5 0Preps) or 6ml (250 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve..

【Protocol 】

This protocol is suitable to extract DNA from insect tissue sample <10 mg.

1. Cut insect tissue samples into into small pieces (as small as possible), transfer into a new 1.5ml centrifuge tube.

2. Add **250µl Buffer ITL and 20 µl Proteinase K to the sample,** mix throughly by vortex or inverting. Incubate at 55°C for 6 hours or overnight, turn over the tube from time to time to make sample lyse completely. Or put in a shaking water bath.

   Note: cut the sample into small pieces can reduce the digestion time. Liquid nitrogen grinding, mechanical homogenizer, glass homogenizer, and bead mill can also reduce the digestion time of tissue samples. For hard lyse insect samples, it can help to increase the digestion process by adding Proteinase K in two parts. And the digestion time depends on the type of sample and the homogenization results.

3. (Optional) Centrifuge at 13,000 x g for 3 minutes, transfer the supernatant into a new 1.5ml centrifuge tube.

   Note:This step is necessary if the lysis reagent is not clear or can see undigested particles apparently.

4. Add 500µl Buffer IL to the sample. Vortex for 30 seconds. .

5. Insert a HiPure DNA Mini Column I into a 2ml Collection Tube. Carefully apply the mixture (including sediments) into the column, centrifuge at 10,000 x g for 30~60 seconds.

6. Discard the filtrate, insect the column in to collection tube. Add 600µl Buffer GW1 to the column, centrifuge at 10,000 x g for 30~60 seconds.

7. Discard the filtrate, insect the column in to collection tube. Add 600µl Buffer GW2 to the column, centrifuge at 10,000 x g for 30~60 seconds

8. Discard the filtrate, insect the column in to collection tube. Centrifuge at 13,000 x g for 3 minutes.

9. Insert the column to a new 1.5ml centrifuge tube (not provided). Add 15~30µl Buffer AE or Buffer TE (pre-heated to 55°C) directly onto the middle of the membrane. Let sit for 2 minutes, and then centrifuge at 13,000 x g for 1 minutes.

10. Discard the column, store DNA at 2~8°C. For long time storage, store at -20°C.

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.