Transiom Insect gDNA kit

Cat No: TIDK-50

Contents

Introduction

Insect DNA Kit is designed for efficient recovery of genomic DNA from insects, arthropods, roundworms, flatworms and etc. The method is suitable for fresh as well as frozen insect samples. Insects are ground in 1X PBS using a mortar and pestle and are subjected to lysis by Proteinase K in a chaotropic salt solution. Following lysis is the binding of DNA to the silica gel membrane of the Spin Column to yield purified DNA. The DNA is isolated and purified using our Fast-Spin column technology and is ideal for downstream molecular based applications including PCR, endonuclease digestion, array, genotyping, etc. PCR inhibitors are effectively removed during the purification process.

Storage and stability:

All components of the Insect kit are stable for at least 12 months at room temperature from date of purchase. RNAse A and Proteinase K should be stored at -20°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer TIW. It is possible to dissolve such deposits by warming the solution at 50°C, though we found that they do not interfare with overall performance.

Before Starting

- Read carefully all manual instructions before starting.
- Add 1.0 ml Nuclease Free Water in Proteinase K and RNAse A.
- Proteinase K and RNAse A should be store at -20°C.

Product components and Storage conditions:

Product	TIDK-50	Storage
Preps	50	-
10X Buffer PBS	6.0 ml	RT
Buffer TIL	45.0 ml	RT
Buffer TIW	15.0 ml	RT
Buffer TW I	10.0 ml	RT
Elution Buffer	10.0 ml	RT
Proteinase K	20 mg	-20^{0}C
RNAse A	20 mg	-20^{0}C
TransPure™ Column	50 nos.	RT
Collection Tubes	50 nos.	RT
User manual	1	-

Important:

- Dilute **Buffer TIW** with **absolute ethanol** as follows and store at room temperature:

  Buffer TIW: Add 15 ml absolute (96%-100%) ethanol per bottle

- Dilute **Buffer TW I** with **absolute ethanol** as follows and store at room temperature:

  Buffer TW I: Add 40 ml absolute (96%-100%) ethanol per bottle.

- Dilute **Buffer 10X PBS** with **Nuclease Free Water** as follows and store at room temperature:

  10X Buffer PBS: Add 54 ml **Nuclease Free Water** per bottle

- Add **1 ml Nuclease Free Water** in **Proteinase K** and **RNAse** containing vial.

ADDITIONAL MATERIALS REQUIRED:

- Absolute (96%-100%) ethanol (Equilibrated at room temperature).
- Microcentrifuge
- Microcentrifuge tubes (1.5-ml)
- Water bath Equilibrated to 60°C.
- Sterile mortar and pestle

Insect gDNA Kit Protocol:

1. **Homogenize up to 100 -125 mg insects in 900 µl of 1X PBS (Ice Chilled) using a mortar and pestle. Transfer the entire homogenize in a 2.0 ml microcentrifuge tube.**
2. **Add 20 µl of Proteinase K and 20 µl of RNAse A to the eppendorf tube.**
3. **Add 750 µl of Buffer TIL and Mix thoroughly by vortexing for 30 seconds.**

   Note: Add adequate volume of Nuclease Free Water as mentioned in 10X PBS buffer, Proteinase K and RNAse A.
4. **Incubate at 60°C for 30-35 minutes either on thermo mixer or water bath or dry bath.**

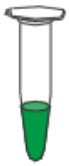
Briefly invert the tube once during incubation.

5. After incubation centrifuge entire lysate centrifuge 5000 rpm for 1 minute. Carefully aspirate supernatant without any debris and transfer into new 2.0 ml tube.
6. Add 350 µl of 96-100% ethanol to the supernatant. Mixed by inverting a tube 15- 20 times.
7. Transfer entire lysate to the DNA spin column, and centrifuge at 12,000 rpm for 1 min. Discard the flow through liquid.
8. Repeat above step, until the entire sample has been processed and retain column for further processing.
9. Place the column into the same collection tube. Add 500 µl of Buffer TIW. Centrifuge at 12,000 rpm for 1 min. discard the flow through.
10. Place the column into same collection tube. Add 500µl of TW I. Centrifuge at 12,000 rpm for 1 min. discard the flow through.
11. Place empty DNA spin column, with the lid open into the same collection tube and centrifuge at 14,000 rpm for 2 min.
12. Place the column into a new sterile 1.5 ml eppendorf tube, add 30 µl preheated Elution buffer. Incubate at room temperature for 5 min. (perform this step twice 30 µl + 30 µl = 60 µl)
13. Centrifuge 10,000 rpm for 1 min to elute pure insect gDNA. The first elution normally yields 60-70 % of DNA bound. A second elution with another 30 µl buffer will yield another 20 % of the DNA.

    Note: Elution volume may vary as per downstream process

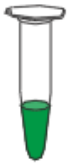
14. Discard the Column, and save elute. Do not reuse binding columns or collection tubes.

FLOW CHART



Preparation of samples

- Take 100-125 mg insect sample.
- Crush into 900µl in chilled 1XPBS.



Lysis

- Add 750µl TIL and mixed with 20µl of Proteinase K and 20µl of RNAse A

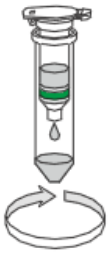
Incubation & Phase Separation

- Incubate at 60^0C for 30-35 min

Binding to silica column

- Adjust binding of phase with ethanol

Hands on time 40 mins



Washing

- Washing twice with wash buffer TIW and TW I
- Dry column

Elution of Pure DNA

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer TIL and incubate for specified time at 60°C. It may be necessary to extend incubation time by 10 min.
	Sample is too high	If using more than 150 mg of insect, increase volume of Proteinase K, RNASe, Buffer TIL, 1X PBS and Ethanol. Pass aliquots of lysate through one column successively.
Low DNA Yield	Clogged Column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 70°C for 5 min with elution buffer may increase yields.
	Improper washing	Wash buffer TIW, TW I concentrate must be diluted with ethanol.
Low A260/280 Ratio	Extended centrifugation during elution	Resin from the column may be present in elute and effect the OD absorbance. Avoid centrifugation at speed higher than 12,000 rpm.
	Poor cell lysis due to incomplete mixing with buffer TBL	Repeat the procedure, this time making sure to vortex the sample with Buffer TIL immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer TBL	Mix thoroughly with buffer TIL.
	No Ethanol added to wash buffer concentrate.	Dilute Wash buffer TIW, TW I with the indicated volume of absolute ethanol before use.
Eluted Material has Red or Brown Color	Sample volume is too high	Reduce sample volume and Proceed with protocol.

Limited Use and Warranty

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