One-Step Insect DNA Purification Kit

Invasive insects can devastate ecosystems and cost billions of dollars in management costs and lost revenue each year. Rapid detection is a vital step in preventing the spread of invasive insects, but detection capabilities must be improved for bulk collections such as those from sticky traps. High-quality DNA extraction from insects is required for reproducible DNA analysis using quantitative PCR, genotyping by sequencing (GBS), or next-generation sequencing (NGS). Standard procedures for extracting insect DNA can be exceedingly Time consuming and labor intensive. Specific applications like plant genotyping demand a lengthy DNA extraction method. For example, traditional plant DNA extractions need a 4-hour to overnight digestion with proteinase K, followed by phenol: chloroform extraction and alcohol precipitation. Other techniques, such as silica bind and elute kits, have recently become available. Those techniques are based on positive chromatography purifying selection. High chaotropic salt concentrations, such as guanidine hydrochloride, bind DNA to silica. The silica column or beads is washed with a salt/ethanol solution after nucleic acid binding to eliminate additional biomolecules from the sample. Finally, the column or beads is eluted using Tris elution buffer or water to remove the pure DNA. Such bind-wash-elute procedures are time-consuming, requiring multiple washing and spinning steps. Repetitive spin steps can cause considerable DNA loss (20-40%) and shearing. Furthermore, chaotropic salts and other impurities can easily pass through the eluted DNA or RNA, compromising ultimate purity and quantification as well as downstream enzymatic activities like PCR.

BcMag™ One-Step Insect DNA Purification Kit allows rapid, column-free extraction of genomic DNA from fresh, frozen, or stored insect specimens such as mosquitoes, bees, lice, ticks, and D. melanogaster The kit uses our unique proprietary magnetic beads and buffers to efficiently lyse cells and remove all impurities simultaneously in an aqueous buffer, leaving the DNA untouched. The procedure employs mild lysis conditions, avoiding harsh conditions such as alkaline lysis and toxic chemicals for lysing cells to maintain DNA integrity and the time-consuming cleanup of organic solvent from the sample. Furthermore, the magnetic beads eliminate PCR inhibitors (Fig.1) from samples in a single step without DNA extraction. It increases DNA integrity, boosts nucleic acid yields, and minimizes DNA loss caused by typical DNA purification techniques' time-consuming "bind-wash-elute" procedure. Following sample lysis, the straightforward one-step purification technique enables simultaneous processing of >96 samples and produces pure DNA in less than 30 minutes. Purified genomic DNA has the highest integrity and can be used in various downstream applications such as qPCR.

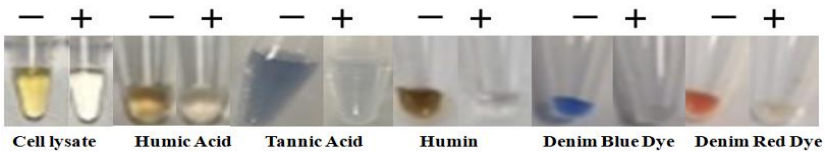

Fig.1 PCR inhibitor removal

Workflow (Fig.2)

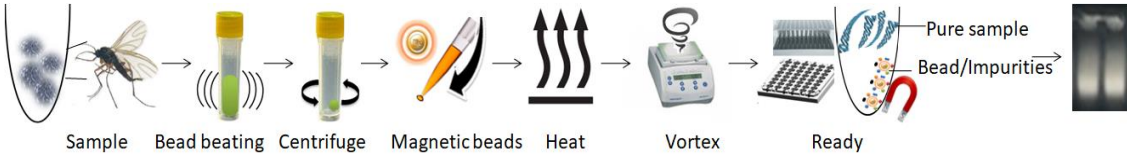

Fig.2 Workflow of one-step insect DNA Purification kit

1. Use bead beating to disrupt the sample in a bead beater.
2. Centrifuge and transfer the supernatant to a new tube.
3. Mix the samples with the magnetic beads and proteinase K and heat to lyse the cells.
4. Vortex beads to capture PCR inhibitors.
5. Remove the beads with a magnet.
6. Aspirate the supernatant containing the pure ready-to-use DNA.

Performance

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The purified DNA is ready for downstream applications, such as PCR, qPCR, single-nucleotide polymorphism (SNP), short tandem repeat (STR) genotyping, genotyping, next-generation sequencing (NGS, veterinary genotyping, etc.

Features and Advantages:

- Rapid and efficient purification protocol: without prior DNA isolation for subsequent use in direct workflows, No liquid transfer, and One-tube.
- Ultrafast: Process 96 samples in less than an hour.
- Highest nucleic acids recovery rates: Minimal loss of DNA during extraction
- Effectively cell lysate cleanup and removes inhibitors: polyphenolic compounds, humic/fulvic acids, acidic polysaccharides, tannins, melanin, heparin, detergents, denim dyes, divalent cations such as Ca^{2+}, Mg^{2+}, etc.
- Cost-effective: Eliminates columns, filters, laborious repeat pipetting, and organic reagents.
- High throughput: Compatible with many different automated liquid handling systems.

Handling and Storage: Store the kit components according to the table below on arrival.

Products

Components	Storage	50 preps, Cat # AM-101	100 preps, Cat # AM-102
BcMag™ U-DNA Beads	4°C	2.5 ml	5.0 ml
10x Lysis Buffer (100mM Tris-HCl, PH 9.0)	4°C	0.6 ml	1.2 ml
Proteinase K	-20°C	12.5 mg	25 mg
DTT(1M)	-20°C	15.4 mg	30.8 mg
Proteinase K Suspension Buffer	4°C	1.0 ml	2.0 ml

PROTOCOL

The following protocol is an example. The protocol can be scaled up or down as needed.

Notes

- DNA Yield: Varies (depends on sample size and type)
- DNA Size: Varies (depends on the quality of starting material
- Since there is no concentration step in the protocol, the concentration of the nucleic acid depends on the quality and quantity of the sample used.
- Quantification of the nucleic acids: Use only fluorescence methods such as qPCR, Qubit, and Pico Green.
- OD260 methods such as Nanodrop and UV-spectrophotometry are not-suitable.
- For long-term storage, store the extracted nucleic acids at -20°C.

Materials Required by the User

Item	Source
Magnetic Rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• BcMag™ Rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • BcMag™ Rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • BcMag™ Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • BcMag™ Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)
BcMag™ 96-well Plate Magnetic Rack.	• BcMa™ 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible Racks (Blioclone, Cat#: MS-06)
Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Addition items are required if using 96-well PCR plates/tubes	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the user should optimize the Time and speed, and the mixer should be: Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529

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Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Tube Holder 1.5/2.0 mL, for 24 × 1.5 mL or 2.0 mL	Eppendorf, Cat#: 022674048
Smart Mixer, Multi Shaker	BenchTop Lab Systems, Cat#:5353000529
1.5/2.0 mL centrifuge tube	
96-well PCR Plates or 8-Strip PCR Tubes	
PCR plates/tubes ** ***IMPORTANT!*** If using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates must be ≥2.5mm.	

Shipping conditions: At ambient temperature

A. Sample preparation

Sample material lysis and disruption

The sample must be completely disrupted to obtain maximum DNA yields. We recommend the Bead Beating method for sample material lysis and disruption. Bead beating is an effective method that disrupts nearly any biological sample to release the DNA, RNA, and proteins found within the cells. Samples are placed in tubes with grinding beads and subjected to high-energy mixing. The beads make contact with the sample, eventually breaking it down at the cellular level and releasing subcellular contents. Typically, the samples are centrifuged, and the lysate retrieved from above the beads.

Instrument

- Beadblaster 24 microtube homogenizer (Benchmark Scientific, Catalog No. D2400)
- Vortex-Genie 2 (Fisher Scientific, Catalog No.50-728-03)
- MN Bead Tube Holder for Vortex-Genie 2 (Takara, Catalog No. 740469)
- FastPrep® homogenizer (MP Biomedicals)

General recommendations for properly homogenizing samples

- Do not overfill tubes. The combined volume of the sample, beads, and buffer should never be more than half the size of the tube. Less is more.
- The sample and buffer volume should be double that of the beads.
- Do not use detergents since the foaming will obstruct the movement of the beads.
- If sample warming is an issue, process the sample in short bursts. •Solid tissues should never be more than 1/20th the volume of the disruption tube, i.e, no more than 100 mg tissue in a 2 ml tube.

The user must select the optimal disruption period based on sample type, sample amount, vortex frequency, and liquid volume in the tube according to the manufacturer's instructions.

- When possible and appropriate, cut the sample into small pieces to facilitate processing.
- Avoid overloading the sample tube to allow efficient mixing of Lysis Mix with the sample.

Sample	Example sample input
Insect samples (Fresh or frozen) Note: For ethanol-preserved insect samples, the ethanol must be completely evaporated.	Method 1 1. Add 10 mg sample to a 2 ml lysis tube containing appropriate grinding beads. Add 750** µl of 1x lysis buffer to the tube and cap tightly. (**Note:** Add 3 µl of 10 mM DTT to 100 µl of 1x lysis buffer immediately before use.) Follow the manufacturer's instructions to process the sample. 2. Centrifuge in a microcentrifuge at ≥10,000 x g for 5 minutes 3. Transfer 5-10 µL supernatant to each well of PCR plate or PCR tube (using 96-well PCR plates/tubes method).
	Method 2 1. Grind 1 mg sample with liquid nitrogen.

B. Premix Beads solution Preparation

IMPORTANT!

1. Before pipetting, shake or Vortex the bottle to completely resuspend the Magnetic Beads.
2. Do not allow the magnetic beads to sit for more than 2 minutes before dispensing.
3. Proteinase K preparation: Provide protease K as lyophilized powder and dissolve at a 20 mg/ml concentration in Proteinase K Suspension Buffer. For example, 12.5 mg dissolved in 625 µl of Proteinase K Suspension Buffer. Divide the stock solution into small aliquots and store at -20°C. Each aliquot can be thawed and refrozen several times but should then be discarded.
4. DTT solution preparation: Provide DTT as powder and dissolve at a concentration of 1M in ultrapure water. For example, 15.4 mg dissolved in 100µl ultrapure water. It is stable for years at -20°C. Prepare in small aliquots, thaw it on ice, and use and discard. Store them in the dark (wrapped in aluminum foil) at -20°C. Do not autoclave DTT or solutions containing it. Avoid multiple freeze-thaw cycles.
5. Dilute DTT to a concentration of 10 mM from stock with ultrapure water and use it immediately. Discard unused DTT solution.
6. Prepare a fresh Master Mix following Table 2 for the number of samples to be processed, plus 10% more (e.g., if you have 10 samples, prepare Master Mix for 11). Add the following components to the reservoir.

Table 2. Premix Beads solution

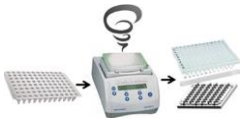
Component	One well (100 µL reaction volume)
BcMag™ U-DNA Beads	50 µL
10x Lysis Buffer	10 µL
Proteinase K (20mg/ml)	12.5 µL
DTT (10 mM)	3 µL
Sample	x
ULTRAPURE WATER	x
Total	100 µL

C. Isolation procedure

IMPORTANT!

- Pipet up and down premix beads solution in a reagent reservoir until the solution is homogeneous before dispensing.
- Do not allow the magnetic beads to sit for more than 5 minutes before dispensing.)

1. Transfer 100µl premix beads solution to the sample to a new well of 96well PCR plate or 0.2ml PCR tube and add the sample.
2. Mix the sample well by Vortex or pipetting.
3. Place the PCR plate/tube into a thermocycler and incubate at:
   a. 65°C for 15 minutes
   b. 80°C for 10 minutes
4. Remove the PCR plate/tube from the thermocycler and then mix the sample with beads by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 5 minutes (see picture).



5. Centrifuge at 3500 rpm for 5 minutes.
6. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
7. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. The sample is ready for downstream applications. Using 1-5 ul in a 25µl of qPCR reaction.

D. Troubleshooting

Problem	Probable cause	Suggestion
Low DNA/RNA Recovery	Poor starting sample material.	• Use better quality of the sample. • Add more samples

Ct value delays	Too many PCR inhibitors in the sample.	1. Add 25-50 µLBcMag™ U-DNA Beads to the extract solution and mix by slowly pipetting up and down 20-25 times, or Vortex the sample at 2000 rpm for 5 minutes. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear. 2. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. Using 1-5 ul in a 25µl RT-PCR or qPCR. The sample is ready for downstream applications.
	Recovery DNA is so low.	• Use better quality of the sample. • Add more samples.

Related products

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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