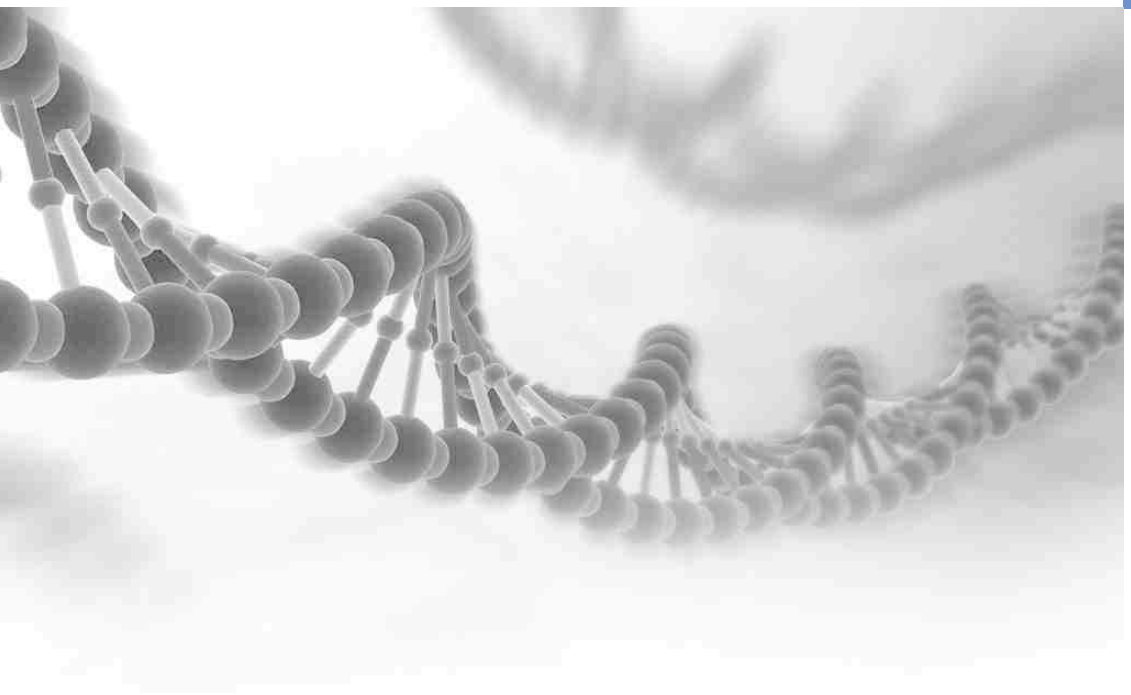
GENETIX BRAND

HANDBOOK

Nucleo-pore®

Nucleo-pore® Insect DNA Extraction Mini kit



☐ **Nucleo-pore® Insect DNA Extraction Mini Kit** NP-6106D 50 Preps

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Nucleo-pore®

Table of Contents

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COMPONENTS

KIT CONTENTS

Nucleo-pore® Insect DNA Extraction Mini Kit

Cat. No.	NP-6106D
No. of Preps.	50
Lysis Buffer ID1	40ml
Lysis Buffer ID2	100ml
Pre- Wash Buffer PWI*	15 ml
Wash Buffer WID*	50 ml
Elution Buffer EID	10ml
Thrashing Bead Lysis tube	50
Fast spin ID Column	50
Fast spin ID Filter	50
Collection Tube	150
Handbook	1

*See Preparation of Storage of Reagents

Reagents, consumables and equipments not provided with the kit

96 – 100% ethanol
Beta-mercaptoethanol to 0.5%(v/v)
1.5 ml microcentrifuge tubes
Disposable pipette tips
Manual pipetts
Centrifuge
Vortex
Equipment for sample disruption and homogenization
Personal protection equipment (e.g., lab coat, gloves, goggles

SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS).

INTRODUCTION

Principle and Procedure

Nucleo-pore Insect DNA Extraction Kit is a simple & fast bead-beating method designed to isolate up to 25 µg total high quality DNA (e.g., genomic, viral, mitochondrial) from small amounts of fresh, frozen or stored insect specimens including mosquitoes, bees, lice, ticks, and D. melanogaster. The procedure is easy and can be completed in as little as 15 minutes: samples are added directly to a ThrashingBead Lysis Tube and rapidly and efficiently lysed by bead beating without using organic denaturants or proteinases. 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. Also, the procedure is compatible with mammalian tissues, whole blood, and cultured cells.

Specifications of Nucleo-pore® Insect DNA Extraction Kit

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Preparation and storage of Reagents

Kit can be used for up to one year from date of purchase. Every lot of the kit is tested to ensure consistent and high quality results.

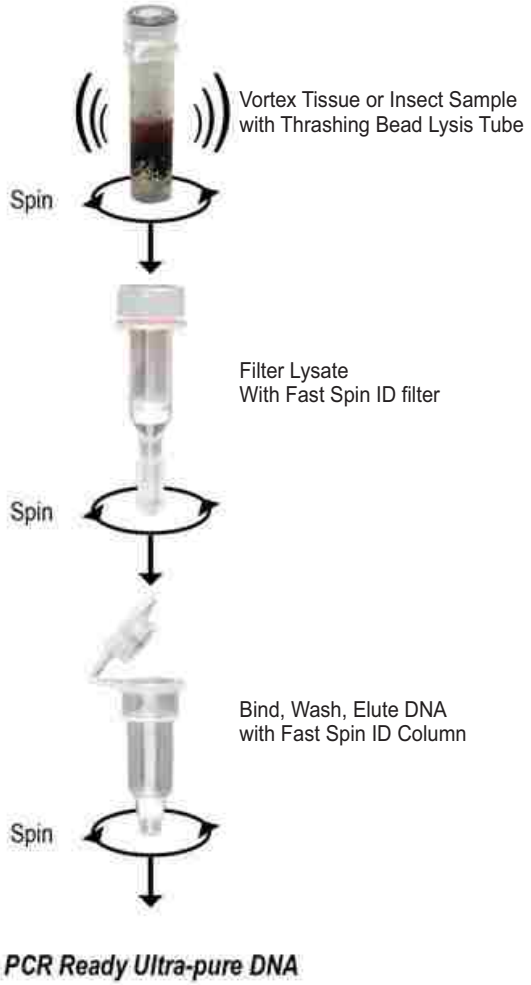
Lysis Buffer ID2

For optimal performance, add beta-mercaptoethanol (not provided with the kit) to the Lysis Buffer ID2 to a final dilution of 0.5%(v/v) i.e., 500 µl per 100 ml.

Pre-Wash Buffer PWI

A precipitate may have formed in the Pre-Wash Buffer PWI during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37ºC for 30 minutes and mix by inversion. DO NOT MICROWAVE.

FLOWCHART



PROTOCOL
Protocol For Insect DNA Purification

Things to do before starting

· Check whether BME was added to the Lysis Buffer ID2 as per instructions
Note: if the Fast Spin matrix is dry, add 400-600 µl water prior to prepping the filter.

PROCEDURE

1. **Take up to 0. 50 grams of sample in a fresh Thrashing Bead Lysis Tube (provided), to it add 750 µl Lysis Buffer ID1 Ensure the lid is tightly closed in order to prevent leakage.**
   Note Generally, no more than 50 mg tissue should be sampled, for larger samples will exceed the DNA binding capacity of the spin column .Up to 400 µl of whole blood or up to 8.5 x10^6 cells suspended in 200 µl PBS can also be sampled.

2. **Secure the Thrashing Bead Lysis tube in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 5 minutes.**
   Note: Processing time may be as little as 40 seconds when using high speed cell disrupters. Alternatively, a standard bench top vortex can be used although the overall DNA yield may be lower.

3. **Centrifuge the Thrashing Bead Lysis Tube in a microcentrifuge at ≥ 10,000 x g for 1 minute.**

4. **Transfer 400µl supernatant to a Fast Spin ID1 Filter placed in a collection Tube and centrifuge at 7,000 x g for 1 minute.**
   Note: Ensure to snap off the base of the Fast Spin Filter prior to use.

5. **Add 1,200 µl of Lysis Buffer ID2 to the filtrate in the Collection Tube from Step 4.**

6. **Transfer 800 µl of the mixture from Step 5 to Fast Spin ID Columns in a collection Tube and centrifuge at 10,000 x g for 1 minute.**
   Note: The loading capacity of Fast Spin ID Columns is 800 µl.

7. **Discard the flow through from the collection tube and repeat Step 6 with the remaining mixture from Step 5.**

8. **Add 200 µl Pre-Wash Buffer PWI to the Fast Spin ID Columns in a new collection tube and centrifuge at 10,000 x g for 1 minute.**

9. **Add 500 µl Wash Buffer WID to the Fast Spin ID Columns and centrifuge at 10,000 x g for 1 minute.**

10. **Transfer the Fast Spin ID Columns to a clean 1.5 ml microcentrifuge tube and add 50 µl (25 µl minimum) Elution Buffer EID directly to the column avoid wetting the rim. Allow it to stand for a minute then centrifuge at 10,000 x g for 30 seconds to elute the DNA.**

11. **The purified DNA is now suitable for downstream applications.**

TROUBLE SHOOTING GUIDE

Poor quality DNA

Possibile Cause
- *Buffers and Reagents not re-constituted properly*

Suggestion
- Reconstitute buffers solution as per instructions.

Possibile Cause
- Insufficient cell lysis

Suggestion
- Vortex the mixture vigorously immediately after addition of Lysis Buffer ID1 .

Possibile Cause
- RNA Contamination

Suggestion
- Add 20ul RNase A solution (20 mg/ml) before addition of Lysis Buffer ID1 if RNA free DNA is required.

Possibile Cause
- Samples inappropriate.

Suggestion
- Use recommended amount of starting material

Suboptimal performance of genomic DNA in enzymatic reactions

Possibile Cause
- Ethanol not completely removed

Suggestions
- Make sure to remove all of ethanol before eluting the DNA. If required add a dry spin after Step 9 at 10,000 x g for 3 minutes

Possibile Cause
- Co-purification of inhibitory substances

Suggestions
- Use EDTA free elution buffer. It is recommended to use the Elution Buffer EID provided with the kit.

Low DNA yield

Possibile Cause

- Inefficient homogenization of samples

Suggestions

- Repeat protocol using new samples and ensure complete homogenization

Possibile Cause

- Inefficient cell lysis due to insufficient mixing of the sample with Lysis Buffer ID1

Suggestions

- Repeat the DNA purification procedure with a new sample. Vortex the mixture vigorously immediately after addition of Lysis Buffer ID1.

Possibile Cause

- Suboptimal elution of DNA from the column

Suggestions

- Preheat Elution Buffer EID to 70 °C before elution. Apply Elution Buffer EID directly onto the center of the silica membrane. Check the pH of Elution Buffer EID as elution efficiency decreases dramatically if elution is performed with buffers of pH < 7.0. It is always recommended to use the Elution Buffer EID supplied with the kit.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
DNASure® Tissue Mini Kit	50 preps	NP-61305
DNASure® Plant Mini Kit	50 preps	NP-79105
DNASure® Plant Mini Kit	250 preps	NP-79107
DNASure® Plant Midi Kit	20 preps	NP-78153
DNASure® Plant Maxi Kit	10 preps	NP-78164
DNASure® Blood Mini Kit	50 preps	NP-61105
DNASure® Blood Mini Kit	250 preps	NP-61107
DNASure® Blood Midi Kit	20 preps	NP-61184
DNASure® Blood Maxi Kit	10 preps	NP-61193
DNASure® Blood FastPure Kit	50 preps	NP-62205
DNASure® Blood FastPure Kit	250 preps	NP-62207
SureSpin® Plasmid Mini Kit	50 preps	NP-37105
SureSpin® Plasmid Mini Kit	250 preps	NP-37107
SureSpin® Plasmid FastPrep Kit	50 preps	NP-47105
SureSpin® Plasmid FastPrep Kit	250 preps	NP-47107
SureSpin® Buffer Set*	1	37107-BS
SurePrep® Plasmid Mini Kit	20 preps	NP-15123
SurePrep® Plasmid Mini Kit	100 preps	NP-15125
SurePrep® Plasmid Midi Kit	20 preps	NP-15143
SurePrep® Plasmid Midi Kit	100 preps	NP-15145
SurePrep® Plasmid Maxi Kit	10 preps	NP-15161
SurePrep® Plasmid Maxi Kit	25 preps	NP-15162
SurePrep® Plasmid Mega Kit	5 preps	NP-15183
SurePrep® Plasmid Giga Kit	5 preps	NP-15191

***SureSpin® Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin® Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

Product Warranty

Nucleo-pore® Insect DNA Extration Mini kit components are intended for research purposes only. They are suitable for in - vitro uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the Nucleo-pore® Insect DNA Extration Mini kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of Nucleo-pore® Insect DNA Extration Mini kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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