MB529MPF16 HiPurA® Pre- filled Plates for Insect DNA Extraction

Kit Contents

Product Code	Reagents provided	MB529MPF16
		96 PR
PF16H1	Pre-filled Plates for Insect DNA Purification	6 no
LA1118B	Magnetic Rod Tip	12 no
DS1005A	Magnetic Beads	2 ml
DS0003	RNase A Solution (20mg/ml)	2 ml
DS0013	Proteinase K	2 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH8.5]	7 ml
DS0015	Lysis Solution (AL)	18 ml
DSCA02	HiShredder (in DBCA016 Collection tube)	96 no

Intended Use

Recommended for isolation of DNA from Insects.

Introduction

HiPurA® Pre- filled Plates for Insect DNA Extraction provides the fastest and easiest way to purify DNA for reliable use in amplification technologies. HiPurA® Pre- filled Plates for Insect DNA Extraction can be used for isolation of genomic DNA from a wide variety of samples, but the performance may vary depending on the sample type.

HiPurA® Pre- filled Plates for Insect DNA Extraction

This kit carries out efficient extraction of genomic DNA from wide type of insects. Sample is first lysed under the highly denaturing conditions provided by Lysis Solution to inactivate DNases and to ensure isolation of intact genomic DNA. The DNA purification procedure comprises of three steps viz. adsorption of DNA to the magnetic beads, removal of residual contaminants and elution of pure genomic DNA. The magnetic beads have a high binding capacity and high-quality genomic DNA is obtained from various species. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

Elution

The yield of DNA depends on the sample type and the number of cells in the sample. A single elution with Elution Solution will provide sufficient DNA to carry out multiple amplification reactions.

Storage

HiPurA® Pre- filled Plates for Insect DNA Extraction can be stored at room temperature (15-25°C) for up to 2 year without showing any reduction in performance. We advise a certain storage temperature for the reagents listed below:

- On receipt store Proteinase K (DS0013): at -20°C.
- On receipt store RNase A (DS0003): at 2-8°C.
- On receipt store Magnetic Beads (DS1005A): at 2-8°C.

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Materials needed but not provided

- 55°C water bath or heating block (For Lysate Preparation)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes) TabSpin® 012 (LA1090)
- Insta NX® Mag16 (LA1118)
- Vortex or Thermo mixer or Shaking water bath
- Liquid nitrogen
- Mortar and Pestle
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)

General Preparation Instructions

1. Preheat a water bath or heating block to 55°C. (For Lysate preparation)
2. **Thoroughly mix reagents**

   Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.
3. Ensure that clean & dry Nuclease-free tubes and tips are used for the procedure.
4. Vortex magnetic beads before use.

RNase A enzyme treatment

RNase A is a type of RNase that is commonly used in research. RNase A (e.g., bovine pancreatic ribonuclease A) is one of the sturdiest enzymes in common laboratory usage. It cleaves 3' end of unpaired C and U residues.

Unit Definition for RNase A

One unit of the enzyme causes an increase in absorbance of 1.0 at 260 nm when yeast RNA is hydrolyzed at 37°C and pH 5.0. Fifty units are approximately equivalent to 1 Kunitz unit. It is completely free of DNases and proteases. The specific activity is 90 U/mg.

The product as supplied is stable at room temperature (15-25°C).

Specimen Collection and Handling

Collect insects in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage.

Types of Specimen

Samples: Insects

Sample Preparation

Fresh as well as frozen insect samples can be used to extract DNA by using HiPurA® Insect DNA Extraction Kit. In case of using frozen samples, equilibrate the sample to room temperature (15-25°C). Avoid repeated thawing and freezing of samples since this will lead to reduced DNA yield. Clean the mortar and pestle thoroughly between different samples to prevent cross-contamination.

Insects:

Grind the weighed insect tissue (not more than 50 mg) using clean mortar and pestle in liquid nitrogen to a fine powder. Transfer the tissue powder to a clean capped 2.0 ml microcentrifuge tube (not provided).

Arthropods (and other soft tissue invertebrates):

Grind the weighed insect tissue (not more than 30 mg) using mortar and pestle in liquid nitrogen to a fine powder. Transfer the tissue powder to a clean capped 2.0 ml microcentrifuge tube (not provided).

Procedure

1. **Digest tissue**
   Add 180 µl of Lysis Solution (AL) (DS0015) and 20 µl of the Proteinase K solution (DS0013) to the capped 2.0ml microcentrifuge tube containing the insect sample ground in liquid nitrogen **(Refer Sample Preparation)**. Mix thoroughly by vortexing and incubate at 55°C until the insects are completely digested. During the incubation, vortex the tube occasionally to disperse the sample or place in a thermomixer or a shaking water bath. Vortex briefly after the digestion is complete.

   NOTE: Time of incubation and lysis depends on the type of insect sample to be processed. Usually lysis is completed in 1- 3 hours. Samples can also be lysed overnight without being affected adversely. If residual RNA is not a concern, continue with step 2.

   Optional RNase A treatment

   If RNA-free genomic DNA is required, add 20 µl of RNase A Solution (DS0003) and incubate for 2 minutes at room temperature (15-25°C); continue with step 2.

2. **Load lysate onto HiShredder (DSCA02)**
   Add the lysate onto HiShredder placed in an uncapped 2.0 ml collection tube and centrifuge for 2 minutes at 13,000 x g (≈14,000 rpm) at room temperature (15-25°C). **This will be your pre- processed sample**.

Set up processing plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Open the door of Insta NX® Mag16 machine.
3. Select **"MB52916"** program.
4. Remove the seal from the Pre-filled Plates for Insect DNA Purification (PF16H1). Place the Pre-filled Plates for Insect DNA Purification (PF16H1).

   NOTE: Take care while peeling off the seal. Hold the plate firmly by one hand and then peel off the seal by holding it in your other hand without shaking the plate.

5. Add **50µl of Elution Buffer** (ET) [10mM Tris-Cl, pH8.5] into the **6th and 12th column of the Pre-filled Plates for Insect DNA purification (PF16H1).**
6. Add approximately 200µl of the pre- processed sample in the **1st and 7th column of the Pre-filled Plates for Insect DNA Purification (PF16H1).**

7. Add 20µl of Magnetic Beads (DS1005A) in the **1st and 7th column of the Pre-filled Plates for Insect DNA Purification (PF16H1).**

8. Place the Magnetic Rod Tip (LA1118B) by sliding onto the machine.

   NOTE: After placing the rods ensure that the rods are properly fixed on their place.

9. Close the door of Insta NX® Mag16 machine.

10. Click on the **RUN** option on the home screen.

11. After the run is complete, remove Pre-filled Plates for Insect DNA Purification (PF16H1) from the position. Discard Magnetic Rod Tip (LA1118B). Dispense the eluted DNA from column 6 and 12 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017) (not provided). The eluate contains pure DNA.

    NOTE: A small amount of magnetic beads may be observed in the final eluate at the bottom of the plate. Avoid transferring these magnetic beads to your PCR reaction mixture.

Storage of the eluate with purified DNA: The recommended storage temperature for the eluted DNA is -80°C. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA.

Warning

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

The yield of DNA depends upon the type and the volume of starting material used.

Performance and Evaluation

The yield and efficiency of purification is determined by performing Real- Time PCR.

Quality Control

Each lot of HiMedia's HiPurA® Pre- filled Plates for Insect DNA Extraction is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled for Insect DNA Extraction is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Please refer disclaimer Overleaf.

Disposal

User must ensure safe disposal by autoclaving and/ or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail to mb@himedialabs.com.

Symbols

	Manufacturer		Do not use if package is damaged
	Catalogue number		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Contains biological material of animal origin
	Batch code		

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