

## MB587PC16 HiPurA® Pre- filled Cartridges for Nematode DNA Extraction

### Kit Contents

Product Code	Reagents provided	MB587PC16
		48 PR
PF16S1	Pre-filled Cartridges for Nematode DNA purification	48 no
LA1118B	Magnetic Rod Tip	12 nos
DS1005A	Magnetic Beads	1 ml
DS0003	RNase A (20mg/ml)	1 ml
DS0013	Proteinase K	1 ml
DS0040	Elution Buffer (ET) [ 10mM Tris-Cl, pH8.5]	4.5 ml
DS0015	Lysis Solution (AL)	9 ml
DSCA02	HiShredder (in DBCA016 Collection tube)	48 nos

### Intended Use

Recommended for isolation of DNA from nematodes.

### Introduction

HiPurA® Pre- filled Cartridges for Nematode DNA Extraction provides the fastest and easiest way to purify DNA for reliable use in amplification technologies. HiPurA® Pre- filled Cartridges for Nematode 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 Cartridges for Nematode DNA Extraction

This kit carries out efficient extraction of genomic DNA from wide range of samples. 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 Cartridges for Nematode DNA Extraction can be stored at room temperature (15-25°C) for up to 2 years without showing any reduction in performance. We advise a certain storage temperature for the reagents listed below:

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

#### **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)
- Cartridge Holder (LA1118CH)
- Insta NX® Mag32 (LA1096)
- Vortex
- Liquid nitrogen
- Mortar and Pestle
- Polypropylene sealing film (Product Code: PR21)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (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 nematodes 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: Nematodes

## Sample Preparation

Fresh as well as frozen samples can be used to extract DNA by using HiPurA® Pre- filled Cartridges for Nematode DNA Extraction. 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.

### Pre- processing of sample:

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

## Procedure

### 1. Digest sample

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

**NOTE:** Time of incubation and lysis depends on the type of nematode sample to be processed. Usually lysis is complete 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 20µl 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). **This will be your pre- processed sample.**

## Set up processing Cartridges 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 Cartridges for Nematode DNA Purification (PF16S1). Place the Pre-filled Cartridges for Nematode DNA Purification (PF16S1) into the Cartridge Holder (LA1118CH).
5. **NOTE: Take care while peeling off the seal. Hold the cartridge firmly by your one hand and then peel off the seal by holding it in your other hand without shaking.**
6. Add 50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040) into the 6<sup>th</sup> well of the Pre-filled Cartridges for Nematode DNA Purification (PF16S1).
7. Add 200µl of pre- processed sample into the 1<sup>st</sup> well of the Pre-filled Cartridges for Nematode DNA Purification (PF16S1).

8. Add 20µl of Magnetic Beads (DS1005A) in the **1<sup>st</sup> well of the Pre-filled Cartridges for Nematode DNA Purification (PF16S1)**.

9. Place the Magnetic Rod Tip (LA1118) by sliding onto the machine.

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

10. Close the door of Insta NX<sup>®</sup> Mag16 machine.

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

12. After the run is complete, remove the Pre-filled Cartridges for Nematode DNA Purification (PF16S1) from the position. Discard the Magnetic Rod Tip (LA1118). Dispense the eluted DNA from well 6 to a new HiPer<sup>®</sup> Lock Microcentrifuge Tube, 2.0ml (MBLA017) (not provided). The eluate contains pure DNA.

**NOTE:** If small amount of magnetic beads are observed in the final eluate then keep the cartridges along with cartridge holder on Magnetic pad (not provided) for 4-5 minute and collect supernatant carefully without disturbing beads pellet in new collection tube.

OR

Take out eluate in new collection tube and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

**\*NOTE: If you process less than 4 samples at a time please order LA1118B- Magnetic Rod Tip (Pack size- LA1118B-4no/ LA1118B-40no).**

**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<sup>®</sup> Pre- filled Cartridges for Nematode DNA Extraction is tested against predetermined specifications to ensure consistent product quality.

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Please refer disclaimer Overleaf.

## Safety Information

The HiPurA® Pre- filled for Nematode 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.










## 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](mailto: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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### Disclaimer :

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