MB529 HiPurA® Insect DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB529		
		20 Preps	50 Preps	250 Preps
DS0010	Lysis Solution (C1)	6 ml	15 ml	75 ml
DS0015	Lysis Solution (AL)	6 ml	15 ml	75 ml
DS0012	Wash Solution Concentrate (WS)	6 ml	15 ml	75 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	6 ml	15 ml	75 ml
MB086	Proteinase K	10 mg	25 mg	125 mg
DS0003	RNase A Solution (20 mg/ml)	0.5 ml	1.25 ml	6.25 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection tube]	20 nos	50 nos	250 nos
DSCA02	HiShredder (in DBCA016 Collection tube)	20 nos	50 nos	250 nos
DBCA016	Collection tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
PW1139	Collection tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Intended Use

Recommended for isolation of DNA from Insects.

Introduction

HiPurA® Insect DNA Purification Kit provides a fast and easy method for purification of total DNA for reliable applications in PCR, Southern blotting technique etc. The DNA purification procedure using the miniprep spin column comprises of three steps viz. adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic DNA. HiMedia's HiElute Miniprep Spin Column (Capped) format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality DNA is obtained from various species. The genomic DNA obtained is compatible with downstream applications such as restriction endonuclease digestion, PCR and Southern blotting.

HiPurA® Insect DNA Purification Kit

This kit simplifies isolation of genomic DNA from insects, arthropods, roundworms, flatworms etc. with spin column procedure. The method is suitable for fresh as well as frozen insect samples. Insects are ground in liquid nitrogen 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 HiElute Miniprep Spin Column (Capped) to yield purified DNA. Two rapid wash steps remove trace salt and protein contaminants resulting in the elution of high quality DNA in the Elution Buffer (ET) provided with the kit. Typically 50 mg of tissue yields upto 5-15 µg of DNA.

HiElute Miniprep Spin Column (Capped) [DBCA03]

HiElute Miniprep Spin Column (Capped) [DBCA03] is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic

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acid-binding properties of the advanced silica gel membrane and the speed plus versatility of spin column technology to yield high quantity of DNA.

The use of spin column facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. This column eliminates the need for alcohol precipitation, expensive resins and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional DNA isolation techniques. DNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer provided with the kit. The purified DNA is upto 20-30 kb in length and can be used for further downstream applications.

Elution

The yield of genomic DNA depends on the sample type. A single elution with 200 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 200 µl will increase the final DNA concentration, but will reduce the overall DNA yield. The eluted DNA ranges in size up to 20-30 kb and is suitable for direct use in PCR, restriction digestion and Southern blotting applications.

Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of DNA. The $A_{260}–A_{320}$ /$A_{280}–A_{320}$ ratio should be 1.6 - 1.9. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. DNA purified by HiPurA® Insect DNA Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor.

Materials needed but not provided

- 55°C water bath or shaking water bath
- 70°C water bath or heating block
- Liquid nitrogen
- Mortar and Pestle
- Tabletop Microcentrifuge capable of at least 14,000 x g (with rotor for 2.0 ml tubes)
- Ethanol (96 -100%)
- Molecular Biology Grade Water (Product Code: ML024)

Storage

Store the HiPurA® Insect DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 1 year.

General Preparation Instructions

1. Preheat a water bath or shaking water bath to 55°C.

2. Preheat a water bath or heating block to 70°C.

3. **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.

4. Ensure that clean & dry tubes and tips are used for the procedure.

5. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
20	6 ml	18 ml
50	15 ml	45 ml
250	75 ml	225 ml

6. **Reconstitute Proteinase K (MB086)**

The HiPurA® Insect DNA Purification Kit contains Proteinase K (MB086). Intensive research has shown that it is the optimal enzyme for use with the Lysis Solution provided in the kit. It is completely free of DNase and RNase activity. Proteinase K is the enzyme of choice for use with an SDS containing Lysis Solution. The specific activity of the Proteinase K is 33.5 units/mg dry weight.

Resuspend the Proteinase K powder in Molecular Biology Grade Water to obtain a 20 mg/ml stock solution.

Number of Preps	Proteinase K	Molecular Biology Grade Water
20	10 mg	0.5 ml
50	25 mg	1.25 ml
250	125 mg	6.25 ml

The product as supplied is stable at room temperature (15-25°C), upon reconstitution store at -20°C as mentioned in storage instructions.

NOTE: The Proteinase K solution must be added directly to each sample preparation every time. Do not combine the Proteinase K and Lysis Solution for storage.

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).

Centrifugation

All centrifugation steps are carried out in conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with fixed angle rotor. The tubes provided with the kit are compatible with almost all laboratory centrifuges and rotors. All centrifugation steps are performed at room temperature and are given in g, the correct rpm can be calculated using the formula:

$$RPM = \sqrt{RCF/1.118 \times 10^{-5} r}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

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 Purification Spin 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.

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.

Procedure

1. **Digest tissue**
   Add 180 µl of Lysis Solution (AL) (DS0015) and 20 µl of the Proteinase K solution (20 mg/ml) **(Refer General Preparation Instructions)** to 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, 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 insect 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 20 µl of RNase A Solution (DS0003) and incubate for 2 minutes at room temperature (15-25°C); continue with step 2.

2. **Lysis reaction**
   Add 200 µl of Lysis Solution (C1) (DS0010) to the sample. Vortex thoroughly for 15 seconds. A homogeneous mixture is essential for efficient cell lysis. Incubate at 70°C for 10 minutes. Continue with step 3.

3. **Load lysate onto HiShredder (DSCA02)**
   Add the lysate onto HiShredder placed in a uncapped 2.0 ml collection tube and centrifuge for 2 minutes at 13,000 x g (≈14,000 rpm).

4. Transfer the flow-through fraction from step 3 to a new 2.0 ml collection tube (not provided) without disturbing the cell debris pellet.

5. Add 200 µl of ethanol (96-100 %) to the lysate and mix thoroughly by vortexing for 5-10 seconds.

   NOTE: A homogeneous solution is essential. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the column. This precipitate does not interfere with the DNA isolation procedure or with any subsequent application. Do not use alcohols other than ethanol because this may result in reduced yields.

6. **Load lysate onto HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Transfer the lysate obtained from step 5 onto column provided. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.

   NOTE: Use a wide bore pipette tip to reduce shearing of the DNA while transferring contents onto the column. It is essential to apply all of the precipitate to the column. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all the solution has passed through. Centrifugation at full speed will not affect the yield or purity of the DNA.

7. **Wash**
   (Prepare Wash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution to the column and centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

8. Add another 500 µl of diluted Wash Solution to the HiElute Miniprep Spin Column (Capped) and centrifuge at 12,000-16,000 x g (≈13,000-16,000 rpm) for 3 minutes to dry the column. Discard the flow-through. Centrifuge the column for another minute at the same speed if residual ethanol is observed. Discard the collection tube containing the flow-through liquid and place the column in a new 2.0 ml uncapped collection tube.

   NOTE: The column must be free of ethanol before eluting the DNA. The tube can be emptied and re-used for the additional centrifugation step.

9. **DNA Elution:**
   Pipette 200 µl of the Elution Buffer (ET) (DS0040) directly into the column without spilling to the sides. Incubate for 1 minute at room temperature. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to elute the DNA.

   Optional: A second elution can be collected by repeating step 9.

   NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Buffer, then centrifuge. Elution with volumes less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing DNA in water can cause acid hydrolysis.

10. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage.

Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

Warning and Precautions

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

Limitations

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

Performance and Evaluation

Each lot of HiMedia's HiPurA® Insect DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Drosophila	5- 15 µg	1.6-1.9

References:

1. Sambrook, J.,*et al.* Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989)
2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A practical guide (Academic Press, San Diego, CA, 1993)

Troubleshooting guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Spin column is clogged	Sample is too large or improperly lysed	Use smaller quantity of sample in subsequent preparation. The current preparation and clogging can be alleviated by increasing the g-force or spinning for longer time until the lysate passes through the binding column. The yield of genomic DNA reduces.
		Inefficient lysis of insect tissue	The Proteinase K digestion at 55°C can be extended. Invert the sample tube after Proteinase K digestion for a homogenous mixture. The Proteinase K solution must be added directly to each sample preparation every time. Do not combine the Proteinase K and Lysis Solutions for storage.
		Lysate/ethanol mixture is not homogenous	Vortex the tubes for atleast 5-10 seconds in order to obtain a homogenous solution before applying it to the column. If minimally sheared DNA is required for downstream applications like PCR, mix with gentle pipetting or inversion until homogenous, instead of vortexing.

2.	Low DNA yield	DNA elution is improper	Ensure that the DNA elution is in 200 µl of Elution Buffer. To improve the DNA yield incubate for 5 minutes at room temperature (15-25°C) after Elution Buffer is added to the column.
		Ethanol was omitted during binding	Ensure that ethanol is added before loading the sample onto the HiElute Miniprep Spin Column (Capped).
		Eluate contains residual ethanol from the wash	Remove ethanol from the second wash completely before eluting the DNA. Spin for an additional 1minute to dry the membrane completely. In order to avoid the interference of ethanol, always use a fresh collection tube for elution.
		Wash Solution Concentrate was not diluted before use	Ensure that the Wash Solution Concentrate is properly diluted with ethanol as per General Preparation Instructions.
		Use of water instead of Elution Buffer for elution of DNA	Elution Buffer is recommended for optimal yield and storage of the genomic DNA. If water is used instead of the Elution Buffer the pH should be atleast 7.0, to avoid acidic conditions, which may cause acid hydrolysis of DNA when stored for long periods of time. **NOTE:** Only DNase/RNase and Protease free water should be used for eluting DNA.
3.	Purity of the DNA is lower than expected; (A_{260}/A_{280} ratio is low)	Eluate was diluted in water for absorbance measurement	Use either the Elution Buffer provided, or 10 mM Tris-HCl, pH 8.0-8.5.
		Background reading is high due to silica fines	Spin the DNA sample at maximum speed for 1 minute, the supernatant can be used to repeat the absorbance readings.
4.	Purity of the DN is higher tha expected; (A_{260}/A_{280} ratio is too high)	RNA contamination	RNase A treatment can be included in future isolations or the final product can be treated with RNase A Solution and repurified.

5.	Shearing of genomic DNA	Improper handling of genomic DNA	All pipetting steps should be executed as gently as possible. Wide orifice pipette tips are recommended to eliminate shearing of the DNA to a large extent. If the isolated DNA is to be used for PCR, instead of vortexing mix with gentle pipetting or invert until homogenous. This reduces shearing of DNA considerably.
		Sample is old, degraded, or has undergone repeated freeze/ thaw cycles.	Fresh insect samples should be used immediately. Old material may yield degraded DNA in the eluate. Avoid repeated thawing and freezing of samples since this will lead to reduced DNA size.
6.	Downstream applications are inhibited	Traces of ethanol present in the final genomic DNA preparation	After the washing steps, the flow-through should not come in contact with the column. Spin the HiElute Miniprep Spin Column (Capped) for 1 minute at maximum speed (12,000-16,000 x g) if necessary, after emptying the collection tube.
		Salt is carried over in the final genomic DNA preparation	The HiElute Miniprep Spin Column should be transferred to a new 2.0 ml collection tube before adding the wash solutions.

Safety Information

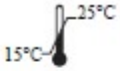
The HiPurA® Insect DNA Purification Kit is for laboratory use only, not for drug, household or other uses. The Lysis Solution (C1) contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing 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.



Storage temperature

Do not use if package is damaged

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