MB581 HiPurA® Shrimp DNA Purification Kit

Kit Contents

Product Code	Reagents provided	20 preps	50 preps	250 preps
DS0015	Lysis Solution (AL)	20 ml	50 ml	250 ml
DS2280	Proteinase K	10 mg	25 mg	125 mg
DS0067	Binding Solution (SB)	16 ml	40 ml	200 ml
DS0019	Wash Solution Concentrate (WSP)	8 ml	20 ml	100 ml
DS0040	Elution Buffer (ET)	6 ml	15 ml	75 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos.	50 nos.	250 nos.
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos.	50 nos.	250 nos.
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 nos.	100 nos.	2 x 250 nos.

Introduction

Shrimp is an important commodity in international trade accounting for 15% in terms of value of internationally traded seafood products. Aquaculture contributes to over 50% of global shrimp production. Shrimp production by aquaculture has been seriously impacted by diseases. The major constraints faced by shrimp aquaculture is the loss due to viral and bacterial diseases like white spot syndrome, yellow head disease, Taura syndrome, *Vibrio* spp., etc. HiMedia's HiPurA® Shrimp DNA Purification Kit provides a fast and easy method for purification of total DNA for reliable applications in PCR technique etc. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

HiPurA® Shrimp DNA Purification Kit

This kit simplifies isolation of DNA from tissues or cells with solution based procedure. Animal tissue (spliced and digested) is subjected to lysis by Proteinase K in a chaotropic salt solution. Following lysis, is the binding of DNA with chilled ethanol to yield purified DNA using HiElute Miniprep Spin Column. 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.

Elution

The yield of genomic DNA depends on the sample type and the number of cells in the sample. Elution with Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. The eluted DNA ranges in size upto 20-30 kb and is suitable for direct use in PCR, restriction endonuclease-digestion, Southern blotting applications and sequencing reactions.

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

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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® Shrimp 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

- Sterile plastic sachets
- Micro-centrifuge (with rotor for 2.0 ml tubes) with cold temperature for centrifugation.
- Chilled Ethanol (96-100%)
- 70% Ethanol
- Molecular Biology Grade Water (Product Code: ML024)
- Micropipettes and Tips

Storage

Store the HiMedia's HiPurA® Shrimp DNA Purification Kit between 15-25°C except certain components as specified on each label. Under recommended conditions, the kit is stable for 18 months.

General Preparation Instructions

1. **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 to cool to room temperature (15-25°C) before use.

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

3. **Dilute Wash Solution Concentrate (WSP) (DS0019) as follows:**

Number of Preps	Wash Solution Concentrate (WSP)	Ethanol (96-100%)
20	8 ml	19 ml
50	20 ml	47 ml
250	100 ml	235 ml

4. **Reconstitute Proteinase K (DS2280)**

   HiMedia's HiPurA® Shrimp DNA Purification Kit contains Proteinase K. 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 (ML024) 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.

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 (15-25°C) 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.

Procedure

1. **Prepare tissue**
   Weigh 100mg of fresh or frozen tissue and mince quickly. If frozen tissue is used, allow it to thaw slightly before slicing but keep on ice in order to protect degradation. Cut the tissue into small pieces as it enables more efficient lysis.

   NOTE: Tissue can be harvested by aliquoting in 2.0 ml collection tubes and flash freezing in liquid nitrogen; these can be stored at -70°C for several months before preparing DNA.

2. **Digest tissue**
   In a sterile plastic sachet, add 800 µL of Lysis Solution (AL) (DS0015) and 20 µl of the reconstituted Proteinase K solution (20 mg/ml) **(Refer to General Preparation Instructions for DNA Extraction)** to the tissue. Homogenize the tissue by grinding it with a pestle. Incubate the sample at room temperature (15- 25°C) for 10 minutes.

3. Transfer the homogenized mixture to a 2 ml micro-centrifuge tube and centrifuge at 5000rpm for 5 minutes.

4. Transfer 150 µl of supernatant to fresh tube and add 450 µL of 100% chilled ethanol. Mix well by vortexing and centrifuge at 10,000 rpm for 5 minutes at 4°C.

5. **Wash**
   Discard the supernatant and re-suspend the pellet with 400 µl of 70% ethanol and centrifuge at 13,000 rpm for 5 minutes at 4°C.

6. Discard the supernatant and dry the pellet. Resuspend the DNA pellet in 100 µl of Elution Buffer (ET) (DS0040).

7. **Binding**
   Add 500 µl of Binding Solution (SB) (DS0067) to the above 100 µl eluate and mix properly by gentle pipetting.

8. **Load onto HiElute Miniprep Spin Column (Capped) (DBCA03)**
   Load the above solution onto the HiElute Miniprep Spin Column (Capped) and centrifuge for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature. Discard the flow-through. Repeat the above step with the remaining sample. Discard the flow-through liquid and reuse the 2.0 ml collection tube (uncapped).

9. **Wash**
   (Prepare Wash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution (WSP) (DS0019) and centrifuge for 1 minute at 6000 x g (≈8000 rpm).

   NOTE: Discard the flow-through and reuse the 2.0 ml collection tube (uncapped).

10. Add another 500 µl of diluted Wash Solution (WSP) to the column and centrifuge for 2 minutes at a maximum speed (≈14,000 rpm). Discard the flow-through liquid and spin the empty column for another minute at the same speed if residual ethanol is observed.

11. **DNA Elution**
    Transfer the column to a new 2.0 ml collection tube (uncapped) and add 100 µl of Elution Buffer (ET) (DS0040) or Molecular Biology Grade Water (ML024) directly onto the center of the column membrane. Centrifuge the tube for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature. Transfer the eluate to a new capped 2.0 ml collection tube for DNA storage.

Storage of the eluate: 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.

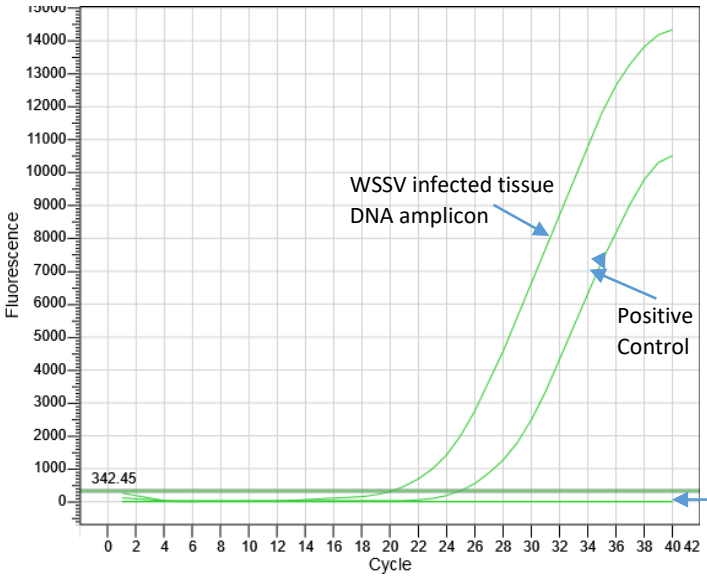
Precautions
Read the procedure carefully before starting the experiment.

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

Quality Control

Type of Sample	DNA Purity
WSSV infected tissue	1.6-1.9

Amplification Data



Sample	Ct value	Fluorescence Value
WSSV infected tissue DNA amplicon	20.29	14000
Positive Control	25.06	10192
Negative Control	N/A	N/A

Image representing probe based real-time amplification data of WSSV DNA extracted using MB581- HiPurA® Shrimp DNA Purification Kit, with C_t values (provided in table). The results completely depend upon sample types.

Troubleshooting guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Low yield of genomic DNA	DNA elution step not performed properly	Ensure that the DNA elution is in 100 µL of Elution Buffer. To improve the DNA yield, incubate for 5 minutes at room temperature (15-25°C) after Elution Buffer is added.
		Ethanol was omitted	Ensure that the ethanol added to the lysate.
		Use of water instead of Elution Buffer for elution of DNA	Elution Buffer is recommended for optimal yields and storage of the genomic DNA. If water is used instead of the Elution Buffer, the pH should be at least 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)
2.	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 as the eluate.
3.	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 cells, tissues. Old material may yield degraded DNA in the eluate. Cells and tissues can be frozen in liquid nitrogen and stored at -70°C until needed.

Safety Information

HiMedia's HiPurA® Shrimp DNA Purification Kit is for laboratory use only; not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Avoid contact with skin, and use eye protection. In case of contact, wash with large amount of water. Seek medical attention. 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.

Symbols

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

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