MB575 HiPurA® Viral DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB575		
		20 Preps	50 Preps	250 Preps
ML116	Resuspension Solution (1X PBS)	6 ml	15 ml	75 ml
DS0010	Lysis Solution (C1)	6 ml	15 ml	75 ml
DS0011	Prewash Solution Concentrate (PW)	8 ml	20 ml	100 ml
DS0012	Wash Solution Concentrate (WS)	4 ml	10 ml	50 ml
DS0042	Elution Solution (RNase- Free Water)	6 ml	15 ml	75 ml
MB086	Proteinase K	10 mg	25 mg	125 mg
DS0192	Carrier RNA	0.2 mg	0.5 mg	2.5 mg
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos	250 nos
DBCA016	Collection Tubes(Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
PW1139	Collection Tubes, Polypropylene (2.0 ml)	40 nos	100 nos	2X 250 nos

Intended Use:

The kit is designed to extract Viral DNA from frozen plasma, serum and other body fluids.

Introduction

HiPurA® Viral DNA Miniprep Purification Kit provides a fast and easy method for purification of total DNA from a wide variety of DNA viruses. 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 Viral 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 Viral DNA is obtained from various species.

HiPurA® Viral DNA Miniprep Purification Kit

This kit simplifies isolation of DNA from fresh, old (more than 24 hours) and frozen plasma, serum and other body fluids with spin-column procedure. Viral DNA purification involves cell lysis, which is achieved by incubation of sample in a solution containing chaotropic ions in the presence of Proteinase K at 56°C. HiElute Miniprep Spin Column (Capped) contains specially developed membranes for optimal binding of Viral DNA. After the initial binding of DNA, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality DNA is finally eluted in the Elution Buffer provided with the kit. Typical yield depends upon the sample volume and virus titer.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED
CE

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800

Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

HiElute Miniprep Spin Column (Capped) [DBCA03]

HiElute Miniprep Spin Column (Capped) is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced 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.

Elution

The yield of viral DNA depends on the sample type and the number of cells in the sample. An elution with 20-150 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reactions. Elution with volume less than 150 µl will increase the final DNA concentration, but will reduce the overall DNA yield.

Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the Viral DNA. Use Elution Solution (RNase- Free Water) 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® Viral 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

- 56°C water bath or heating block
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 - 100%)
- Molecular Biology Grade Water (ML024)

Storage

Store the HiPurA® Viral DNA Purification Kit between 15-25°C. Store the DS0192-Carrier RNA in -20°C temperature on receipt. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw. Under recommended condition kit is stable for 1 year.

General Preparation Instructions

1. Preheat a water bath or heating block to 56°C

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. The reagent should be at room temperature (15-25°C) before use.

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

4. **Preparation of Carrier RNA**

No. of Samples	Carrier RNA	Elution Buffer (RNase free water)
20	0.2 mg	200 µl
50	0.5 mg	500 µl
250	2.5 mg	2.5 ml

NOTE: Dissolve Carrier RNA thoroughly by pipetting. Store carrier RNA at -20°C in aliquots. Do not freeze –thaw aliquots of carrier RNA.

5. **Preparation of Lysis Solution (C1)- Carrier RNA**

No. of Samples	Volume of Lysis Solution (C1)	Volume of Carrier RNA
20	4.4 ml	123.2 µl
50	11 ml	308.0 µl
250	55 ml	1540 µl

NOTE: Concentration of Carrier RNA to be used is 28µg/ml.

Calculate the volume of Lysis Solution (C1)- Carrier RNA as follows:

a X 0.22ml = **b** ml

b ml X 28 µl/ml = **c** µl

where, **a** = number of samples to be processed
b = volume of Lysis Solution (C1) to be added for '**a**' number of samples
c = volume of Carrier RNA to be added to Lysis Buffer (C1)

eg: for 2 number of samples, add 0.44 ml of Lysis Solution (C1) and 12.3µl of Carrier RNA.

6. **Dilute Prewash Solution Concentrate (PW) (DS0011) as follows:**

Number of Preps	Prewash Solution Concentrate (PW)	Ethanol (96-100%)
20	8 ml	12 ml
50	20 ml	30 ml
250	100 ml	150 ml

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

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
20	4 ml	12 ml
50	10 ml	30 ml
250	50 ml	150 ml

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

   The HiPurA® Viral 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 Proteinase K is 33.5 units/mg dry weight.

Resuspend the Proteinase K (MB086) 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; 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 solutions for storage.

Centrifugation

All centrifugation steps are carried out in conventional laboratory 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 Handling and Collection

Collect plasma, serum or other body fluids in a sterile container. Thaw the samples on ice before use. Repeated freeze- thaw of samples should be avoided.

Types of Specimen

Clinical samples: Whole blood, plasma, serum

Procedure

NOTE: If the sample is less than 200 µl, add the Resuspension solution (ML116) to bring the volume upto 200 µl.

1. Add 25 µl of the reconstituted Proteinase K solution (20 mg/ml) **(Refer to General Preparation Instructions)** into 2.0 ml capped collection tube containing 200 µl of plasma, serum or body fluid. Vortex for 10-15 seconds to ensure thorough mixing.

   NOTE: Do not add Proteinase K directly to Lysis Solution (C1)

2. **Lysis reaction**
   Add 200 µl of the Lysis Solution (C1)- Carrier RNA to the sample, vortex thoroughly for 15 seconds to obtain a homogenous mixture. Incubate at 56°C for 15 minutes. **(Refer General Preparation Instructions)**

   NOTE: Concentration of Carrier RNA per sample is 28µg/ml.

3. Centrifuge the samples for 10 seconds to remove droplets formed inside the cap of the collection tubes.

4. **Prepare for Binding**
   Add 250 µl of ethanol (96-100%) to the lysate obtained from the above step for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting. Incubate the lysate for 5 minutes at room temperature (15-25°C)

   NOTE: A homogenous solution is essential.

5. Centrifuge the samples for 10 seconds to remove droplets formed inside the cap of collection tubes.

6. **Load lysate in HiElute Miniprep Spin Column (Capped)** [**DBCA03]**
   Transfer the lysate obtained from step 5 into the spin column provided. Centrifuge at 8,000 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 when transferring contents into the column.

7. **Prewash**
   (Prepare Prewash Solution Concentrate (PW) (DS0011) as indicated in General Preparation Instructions)
   Add 500 µl of diluted Prewash Solution to the column and centrifuge at 8,000 rpm for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

8. **Wash**
   (Prepare diluted Wash Solution Concentrate (WS) (DS0012) as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution to the column and centrifuge at 8,000 rpm for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

9. **Second Wash**
   Add 500 µl of Ethanol (96-100%) to the column and centrifuge at 8,000 rpm for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

10. Place the column in a new collection tube (not provided) and spin the empty column for another minute at 14,000 rpm for 3 minutes to dry the membrane.

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

11. Place the column in new collection tube (not provided) and incubate at 56°C for 3 mins to dry the column.

12. **DNA Elution**
    Pipette 20-150 µl of the Elution Buffer (RNase free water) (DS0042) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at 13,000 rpm for 1 minute to elute the DNA.

    NOTE: DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time (DNA yield would be low). Storing DNA in water may cause acid hydrolysis. To increase the elution efficiency, incubate for 5 minutes at room

temperature (15-25^{0}C) after adding the Elution Buffer (ET), then centrifuge. Elution with volume less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

13. 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 Viral 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

Certified for *in vitro* Diagnostic Use (IVD). 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

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

Performance and Evaluation

Performance of the kit is expected when the kit is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.

Quality Control

Type of sample	PCR amplification
HPV serum sample	Observed

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).

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Poor or low Viral DNA recovery	Carrier RNA is not added to Lysis Solution (C1)	Add carrier RNA to Lysis Solution (C1) before use. **(Refer General Preparation Instructions)**
		Lysis Solution (C1)-Carrier RNA mixture is not homogenous	In order to obtain a homogenous solution, mix thoroughly by gentle pipetting before adding to the Lysis Buffer to the sample.

		DNA elution is improper	Ensure that the DNA elution is in 200 µl of Elution Buffer (RNase free water) as mentioned in step 12. To improve the DNA yield incubate for 5 minutes at room temperature (15-25°C) after it is added to the column.
		Eluate contains residual ethanol from wash	Remove ethanol from the second wash completely before eluting the DNA. Spin for an additional 3 minutes to dry the membrane completely. In order to avoid the interference of ethanol, fresh tube can be used for elution.
		Use of water instead of Elution Buffer for elution of DNA	Elution Buffer (RNase free water) is recommended for optimal yield and storage of the Viral DNA.
3.	Purity of the DNA is lower than expected (A_{260}/A_{280} ratio is less)	Background reading is high due to silica fines	Spin the DNA sample at maximum speed for 1 minute and use the supernatant to repeat the absorbance readings.
		Eluate was diluted in water for absorbance measurement	Use the Elution Buffer (RNase free water) provided with the kit.
		Purification is incomplete due to column overloading or inadequate lysis	Reduce the initial volume of the sample or increase the lysis time while monitoring the lysis visually.
4.	Shearing of Viral DNA	The sample used is very old, degraded or has undergone repeated freeze/ thaw cycles	If the sample is very old, the eluate may yield degraded DNA. For best results, fresh samples should be used or can be stored at 4°C for up to 3 months.

Safety Information

The HiPurA® Viral 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.

Please refer disclaimer Overleaf.

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.

IVD	In vitro diagnostic medical device
CE	CE Marking
15°C–25°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Private Limited, Reg. Off: Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane, (West) 400604, Maharashtra, INDIA. Web: www.himedialabs.com
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu
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