MB582 HiPurA® Viral DNA/ RNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB582		
		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)	6 ml	15 ml	75 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	0.28 mg	0.7 mg	3.5 mg
DS0037	RNA Lysis Solution (HRL)	16 ml	40 ml	200 ml
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, RNA from fresh and frozen plasma, serum, nasopharyngeal swab, oropharyngeal swab, sputum, BAL and other body fluids.

Introduction

HiPurA® Viral DNA/ RNA Purification Kit provides a fast and easy method for purification of total DNA/ RNA from a wide variety of DNA and RNA viruses. The DNA and RNA purification procedure using the miniprep spin column comprises of three steps viz. adsorption of DNA/ RNA to the membrane, removal of residual contaminants and elution of pure Viral DNA/ RNA. 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/ RNA is obtained from various species.

HiPurA® Viral DNA/ RNA Purification Kit

This kit simplifies isolation of DNA/ RNA from fresh, old (more than 24 hours) and frozen plasma, serum and other body fluids with spin-column procedure. Viral DNA/ RNA 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 and RNA. After the initial binding of DNA/ RNA, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality DNA/ RNA is finally eluted in the Elution Buffer provided with the kit. Typical yield depends upon the sample volume and virus titer.

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WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED
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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/ RNA.

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/ RNA isolation techniques. DNA/ RNA 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/ RNA depends on the sample type and the number of cells in the sample. An elution with 20-80 µl of Elution Buffer (ET) will provide sufficient DNA/ RNA to carry out multiple amplification reactions. Elution with volume less than 80 µl will increase the final DNA/ RNA concentration, but will reduce the overall DNA/ RNA 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/ RNA 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.

Concentration, yield and purity of RNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the RNA. Use Elution Solution 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 40 µg/ml of RNA. The A_{260}-A_{320}/A_{280}-A_{320} ratio should be 1.8 – 2.1. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. RNA purified by HiPurA® Viral DNA/ RNA Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of RNA sample (µg/ml) = 40 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)
- RNase – free pipette tips (aerosol barrier recommended)

Storage

Store the HiPurA® Viral DNA/ RNA 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.

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.

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 Nuclease-free tubes and tips are used for the procedure.
4. **Preparation of Carrier RNA**

No. of Samples	Carrier RNA	Elution Buffer (RNase free water)
20	0.28 mg	280 µl
50	0.7 mg	700 µl
250	3.5 mg	3.5 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. **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

6. **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

7. **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.

8. **Preparation of Lysis Solution (C1)- Carrier RNA (FOR DNA)**

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.

9. **Preparation of Carrier RNA –Lysis Solution (HRL) (FOR RNA)**

Number of Preps	Volume of Carrier RNA	Volume of Lysis Solution (HRL)
20	112 µl	11.2 ml
50	280 µl	28 ml
250	1400 µl	140 ml

NOTE: Concentration of Carrier RNA to be used is 10µg/ml

Calculate the volume of Carrier RNA –Lysis Solution (HRL) as follows:

10.

11. **a** X 0.56 ml = **b** ml

b ml X 10 µl/ml = **c** µl

12. where, **a** = number of sample to be processed

b = volume of Lysis Solution (HRL) to be added for '**a**' number of samples

c = volume of Carrier RNA to be added to Lysis Buffer (HRL)

eg: for 2 number of samples, add 1.12 ml of Lysis Solution (HRL) and 11.2 µl of Carrier RNA

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: Body fluids, plasma, serum

DNA

Procedure for DNA Extraction

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.

RNA

Precautions to be taken while handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non- disposable vessels and solutions while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.

2. Use sterile, disposable plasticware and autoclavable pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipments.

3. Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH, 1mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.

4. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240ºC for four or more hours before use. Alternatively, glassware can be treated with DEPC (Diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 min to eliminate residual DEPC.

5. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.

6. Solutions (water and other solutions) should be treated with 0.1% DEPC

Procedure for RNA Extraction

1. Add 140 µl of cell free sample like serum, plasma or body fluid, nasopharyngeal swab, oropharyngeal swab, sputum, BAL to Collection Tube, Polypropylene (2.0 ml).

2. Add 560 µl of Carrier RNA-Lysis Solution (HRL) to the sample. (**Refer to General Preparation Instructions)**. Mix by pulse vortexing for 15 seconds.

1. Incubate for 10 minutes at room temperature (15-25°C).

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

3. **Binding**
   Add 560 µl of ethanol (96-100%) to the sample, mix well by gentle pipetting.

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

5. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Transfer the lysate obtained in step 6 onto the HiElute Miniprep Spin Column. Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through after the spin. Repeat step 7 with the remaining sample. Reuse the collection tube.

6. **First Wash**
   (Prepare Wash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution (WS) (DS0012). Centrifuge at 8,000 rpm for 1 minute. Discard the flow-through. Reuse the collection tube.

7. **Second Wash**

Add another 500 µl of diluted Wash Solution (WS) (DS0012) onto the column. Close the tube gently and centrifuge for 3 minutes at 14,000 rpm to wash the column. Discard the flow-through. Centrifuge for 1 minute at 14,000 rpm to dry the membrane.

8. Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 60-80 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Incubate for 1 minutes at room temperature (15-25°C). Close the tube gently and centrifuge for 1 minute at 8,000 rpm.

9. Transfer the eluate to a new capped 2 ml collection tube for long-term storage.

   Storage of the eluate with purified RNA: The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

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/ RNA 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
Dengue positive 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: (FOR DNA)

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.

Trouble shooting Guide: (FOR RNA)

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiElute Miniprep Spin Column (Capped)	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		Centrifugation temperature is too low	The centrifugation temperature should be 20 – 25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol containing

			lysate to 37°C before transferring it to the column.
2.	Low RNA Yield	Too much of starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		RNA still bound to HiElute Miniprep Spin Column	Repeat RNA elution, but incubate the column for 10 minutes at room temperature with Elution solution (RNase free water) before centrifuging.
		Ethanol carryover	During the second wash with Wash Solution (WS) be sure to centrifuge at ≥8000 x g (≥10, 000 rpm) for 2 minutes to dry the column. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow through otherwise carryover of ethanol will occur. To eliminate any chance of possible ethanol, centrifuge the column for another step minute at full speed.
		No DNase treatment	Follow the optional on-column DNase digestion
5.	RNA does not perform well in downstream experiments	Ethanol carryover	During the second Wash using Wash Solution (WS), be sure to dry the HiElute Miniprep Spin Column membrane by centrifugation at ≥8000 x g (≥10,000 rpm) for 2 minutes to dry the membrane. Following the centrifugation, remove the HiElute Miniprep Spin Column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

Safety Information

The HiPurA® Viral DNA/ RNA 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.

IVD	In vitro diagnostic medical device
CE	CE Marking
25°C 15°C	Storage temperature
	Do not use if package is damaged
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