MB617 HiPurA® All Blood RNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB617	
		20 Preps	50 Preps
DS0037	RNA Lysis Solution (HRL)	16 ml	40 ml
DS0041	Pre Wash Solution (RW1)	18 ml	45 ml
R075	10X RBC Lysis Buffer Solution	24 ml	60 ml
DS0012	Wash Solution Concentrate (WS)	8 ml	20 ml
DS0042	Elution Solution (RNase- Free Water)	2 ml	5 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos
DSCA02	HiShredder (in DBCA016 Collection tube)	20 nos	50 nos
PW146	Micro Centrifuge Tube-B (1.5 ml)	20 nos	50 nos
DBCA016	Collection Tubes(Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos
PW1139	Collection Tubes, Polypropylene (2.0 ml)	40 nos	100 nos

Intended Use

Recommended for isolation of RNA from human blood samples.

Introduction

HiPurA® All Blood RNA Purification Kit provides a fast and easy method for purification of total RNA for RT-PCR and cDNA library construction. The RNA purification procedure using the miniprep spin columns comprises of three steps viz, adsorption of RNA to the membrane, removal of residual contaminants and elution of pure 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 RNA is obtained from various species (eg: human, animals). The RNA obtained is compatible with various downstream applications as mentioned above.

HiPurA® All Blood RNA Purification Kit

This kit simplifies isolation of total cellular RNA from fresh, whole blood with spin-column procedure. The lysis buffer provided in the kit helps in cell disruption and denaturation, samples are centrifuged through a HiShredder which allow fast and simple homogenization of cell lysates without risk of cross-contamination. Ethanol is added to the cleared lysate, which promotes selective binding of RNA to the HiElute Miniprep Spin Column membrane. After the initial binding of RNA, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality RNA is finally eluted in the Elution Solution provided with the kit.

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WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED
CE

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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 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 RNA isolation techniques. 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 RNA depends on the sample type and the number of cells in the sample. A single elution with 30-50 µl of Elution Solution will provide sufficient RNA to carry out multiple amplification reaction.

Concentration, yield and purity of RNA purified

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 this 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

- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes) at 4°C
- RNase -free pipette tips (aerosol barrier recommended)
- Ethanol (70%)
- 14.3 M ß-mercaptoethanol (ß -ME) (MB041)
- Deoxyribonuclease I Solution (RNase-Free) and DNase Digest Buffer (procure from any standard company)
- HiSep™ LSM 1077, Lymphocyte Separation Media (LS001)

Storage

This kit can be stored at room temperature (15-25°C) except certain components as specified on each labels. Under recommended condition kit is stable for 1 year.

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 automatic 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 240°C 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 100ºC 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.

General Preparation Instructions

1. **ß -mercaptoethanol (ß -ME) must be added to RNA Lysis Solution (HRL) before use.**
   ß-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10µl ß-ME per 1ml Lysis Solution. Lysis Solution containing ß-ME can be stored at room temperature for up to 1 month.
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 Eppendorf DNase, RNase free tubes, tips are used for the procedure.
4. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
20	8 ml	24 ml
50	20 ml	60 ml

5. **Dilute 10X RBC Lysis Buffer Solution (R075) as follows:**

Number of Preps	10X RBC Lysis Buffer Solution (R075)	RNase- Free Water
20	24 ml	216 ml
50	60 ml	540 ml

NOTE: Prepare 1X RBC Lysis Buffer Solution in an appropriately sized RNase-Free Glass bottle (not provided) and store at 2-8°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 Handling and Collection

Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the blood sample is at room temperature (15-25°C) before beginning the protocol. After use, contaminated material must be sterilized by autoclaving before discarding.

Types of Specimen

Clinical samples: Whole blood

IMPORTANT
Equilibrate the 1X RBC Lysis Buffer Solution to Room Temperature (15-25°C) before starting.

Procedure

1. Preparation of sample (For Erythrocyte Lysis)
   (Prepare 1X RBC Lysis Buffer Solution as indicated in General Preparation Instructions)
   Mix 1 volume of whole blood with 5 volumes of 1X RBC Lysis Buffer Solution (R075) in Micro Centrifuge Tube-B (1.5 ml) (provided). For example, to 200 µl of whole blood, add 1 ml of 1X RBC Lysis Buffer Solution.

   For optimal results the volume of mixture (Blood + 1X RBC Lysis Buffer Solution) should not exceed ¾ of the volume of the tube to allow efficient mixing. For example, add 5 ml of 1X RBC Lysis Buffer Solution to 1 ml of whole blood, and mix in a tube which has the total volume of ≥8 ml. Whole Blood treated with any common anticoagulant such as heparin or EDTA can be used in this protocol.

   NOTE: Use an appropriate amount of whole blood. Upto 1.5 ml of healthy blood (typically 4000-7000 leukocytes per microliter) can be processed. Reduce amount appropriately if blood with elevated numbers of leukocytes is used. (In this case, also adjust amount of RNA Lysis Solution (HRL) in step 6).

   A common alternative to erythrocyte lysis is HiSep™ LSM 1077, Lymphocyte Separation Media (LS001). In contrast to erythrocyte lysis procedures, HiSep™ LSM 1077 offers a quick and reliable method for the simple isolation of human mononuclear cells and lymphocytes. Mononuclear cells isolated using HiSep™ LSM can be processed with Blood RNA Purification Kit.

2. Incubate for 10-15 minutes on ice. Mix by vortexing briefly 2 times during incubation. The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, incubation time can be extended to 20 minutes.

3. Centrifuge at 400 x g (≈1700 rpm) for 10 minutes at 4°C, and completely remove and discard supernatant.

   NOTE: Leukocytes will form a pellet after centrifugation. Ensure supernatant is completely removed.

4. Repeat lysis step with the cell pellet, by adding 2 volumes of 1X RBC Lysis Buffer Solution per 1 volume of whole blood used in step1. For example, to 200 µl of whole blood, add 400 µl of 1X RBC Lysis Buffer Solution. Thoroughly vortex to resuspend the cells.

5. Centrifuge at 400 x g (≈1700 rpm) for 10 minutes at 4°C, and completely remove and discard supernatant. Incomplete removal of the supernatant will interfere with lysis and subsequent binding of RNA to the HiElute Miniprep Spin Column, resulting in lower yield.

6. **Lysis reaction**
   Add RNA Lysis Solution (HRL) to pelleted leukocytes according to the table below. Vortex or pipet to mix.
   When not using healthy blood, refer to number of leukocytes to determine the volume of RNA Lysis Solution (HRL) required. RNA Lysis Solution (HRL) disrupts the cells. No cell clumps should be visible before you proceed to the homogenization step. Vortex or pipet further to remove any clumps.

   NOTE: Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.

RNA Lysis Solution (HRL)	Whole blood (ml)	Number of leukocytes
350 µl	Up to 0.5	Up to 2x 10^6
600 µl	0.5 to 1.5	2x 10^6 to 1x 10^7

7. Pipet the lysate directly into a HiShredder (DSCA01) placed in a 2 ml collection tube, and centrifuge for 2 min at full speed to homogenize.

   NOTE: If too many cells have been used, after homogenization the lysate will be too viscous to pipet. In this case divide the sample into two aliquots and adjust the volume of each aliquot to 600 µl with RNA Lysis Solution (HRL). Continue the protocol from step 7.

8. **Prepare for binding**
   Add 1 volume (350 µl or 600 µl) of 70% Ethanol to the homogenized lysate and mix by pipetting. Do not centrifuge.

   NOTE: A precipitate may form after the addition of ethanol, but this will not affect the procedure.

9. **Load Lysate in HiElute Miniprep Spin Column (DBCA03)**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column. Close the tube gently, and centrifuge for 1min at ≥8000 x g (≥10,000 rpm). Discard the flow-through.

   NOTE: If the volume exceeds 700 µl, load aliquots successively onto the HiElute Miniprep Spin Column and centrifuge as above. Discard the flow-through after each centrifugation step.

   Optional: On Column DNase digestion
   Generally, DNase digestion is not required since the solutions of this kit efficiently remove most of the DNA without DNase treatment. However further DNase treatment may be necessary for certain RNA applications that are sensitive to small amounts of DNA

(e.g.TaqMan RT-PCR analysis with a low abundant target). DNA can also be removed by DNase digestion.
Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column as indicated above. Instead of continuing with the Pre Wash Solution (RW1) in step 7, follow steps 9a –9d below.

9a. Pipet 350 µl of Pre Wash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 15 sec at ≥8000 x g (≥10,000 rpm). Discard the flow through and reuse the collection tube in step 9c.

9b. Add 10 µl of DNase I Solution to 70 µl of DNase Digest Buffer. Mix by inversion. Do not vortex.

9c. Add 80 µl of DNase I/ Digest Buffer mixture directly onto the HiElute Miniprep Spin Column. Incubate at room temperature for 15 min.

9d. Pipet 350 µl of Pre Wash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 15 sec at ≥8000 x g (≥10,000 rpm). Discard the flow through and continue with the step 11.

Or

Alternatively, residual DNA can be removed by a DNase digestion after RNA isolation

10. **Pre Wash**
Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column centrifuge at ≥8000 x g (≥10,000 rpm) for 15 s. Discard the flow-through. Reuse the collection tube in step 11.

11. **Wash**
Transfer the HiElute Miniprep Spin Column into a 2 ml collection tube. Pipet 500 µl of diluted Wash Solution (WS) **(Refer General Preparation Instructions)**. Close the tube gently, and centrifuge for min at ≥8000 x g (≥10,000 rpm) to wash the column. Discard the flow- through.

12. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin Column. Close the tube gently, and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the membrane.

13. Place the column in a new 2ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at ≥10000 x g (≥13,000 rpm) at room temperature (15-25°C) for 1 minute.

14. **RNA Elution**
Transfer the HiElute Miniprep Spin column to a new 2 ml collection tube. Pipet 30-50 µl Elution Solution (RNase- Free Water) directly onto the HiElute Miniprep Spin column. Close the tube gently, and centrifuge for 1 min at ≥8000 x g (≥10,000 rpm) to elute.

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

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 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	RNA Purity
Human Blood	1.8-2.1

References:

1. Sambrook, J.,et al. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989; pp. 7.3-7.5)
2. Farrell, Robert E., Jr.; RNA Methodologies; 2nd Edition; Academic Press: NY, 1998; pp. 37-53(Cat. No. Z350354)

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiElute Miniprep Spin Column	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).
		RNA still bound to HiElute Miniprep Spin Column	Repeat RNA elution, but incubate the HiElute Miniprep Spin Column for 10 mins 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 2 min at ≥8000 x g (≥10, 000 rpm) 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 minute at full speed.

2.	Little or no RNA eluted	RNA remains on the column	Repeat elution step or preheat the Elution Solution (RNase- Free Water) to 70°C prior to elution.
3.	Low A_{260} / A_{280}	Water used to dilute RNA for A_{260} / A_{280} measurement.	Use 10 mM Tris – Cl, pH 7.5, not RNase free water to dilute the sample before measuring purity.
4.	DNA contamination in downstream experiments	No incubation with Pre Wash Solution (RW1)	In subsequent preparations, incubate the HiElute Miniprep Spin Column for 5 min at room temperature after the addition of Pre Wash Solution (RW1) and before centrifuging.
		No DNase treatment	Follow the optional on-column DNase digestion step.
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 min 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

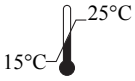
This kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling 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.

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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	08/2027

PIMB617_0/0824 MB617-05

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