RNA Lysis Solution (HRL)

Product Name	Product Code	Kit Packing
RNA Lysis Solution (HRL)	DS0037-200ML	200 ml

Intended Use

Recommended for isolation of RNA from human blood samples.

Introduction: RNA Lysis Solution (HRL) is a buffer solution used for the purpose of lysing blood cells for use in molecular biology experiments. This solution contains a detergent to break up the membrane structures.

Application: RNA Lysis Solution (HRL) is used for isolation of RNA from blood cells.

Properties:

Appearance: Colorless solution
Clarity: Clear and free of particles
DNase & RNase: None detected
Suitability Test: This reagent has been tested and is suitable for isolation of RNA from blood samples.

Storage conditions: RNA Lysis Solution (HRL) has to be stored on receipt at 2 - 8°C. The shelf- life of this solution in 12 months.

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 tissues in a sterile container. Store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the blood sample/ tissues are 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

Procedure

1. Preparation of sample (For Erythrocyte Lysis)
   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.

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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

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). 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.

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 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 solution is expected when the solution 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

Safety Information

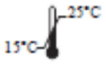
The RNA Lysis Solution (HRL) contains irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

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

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

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HiMedia Laboratories Pvt. Ltd. Reg.office : 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 Email: techhelp@himedialabs.com Website: www.himedialabs.com