For life science research only.
Not for use in diagnostic procedures.

Red Blood Cell Lysis Buffer

Version: 10
Content Version: November 2021

For preferential lysis of red blood cells from human whole blood.

Cat. No. 11 814 389 001 100 ml
50–500 reactions, depending on sample size (1–500 µl)

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Red Blood Cell Lysis Buffer	Ready-to-use solution.	1 bottle, 100 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Red Blood Cell Lysis Buffer	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Sterile, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Autoclaved reaction tubes
- Standard benchtop microcentrifuge
- Rocking platform or gyratory shaker

1.4. Application

The Red Blood Cell Lysis Buffer is used for both DNA and RNA isolation.

- Designed for the preferential lysis of red blood cells from human whole blood, yielding intact white blood cells free of red blood cells for further applications.
  As most blood cells are red blood cells, which lack nuclei and therefore possess no DNA, the lysis and the centrifugation steps concentrate the nucleated white blood cells.
- It is not intended for use with whole blood from any other species.
- Eliminates the need for hazardous organic extractions or chaotropic agents.
- Particularly useful for high-volume research currently requiring Ficoll-Hypaque gradients.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Handling requirements

- Warm the Red Blood Cell Lysis Buffer to +15 to +25°C prior to use.
- Warm blood to +15 to +25°C.
  ⚠ ***Do not use blood that has been frozen and thawed more than three times for any DNA applications.***
  ⚠ ***Do not use blood that has been stored for longer than 1 month for any DNA applications.***
- Perform all centrifugation steps at +15 to +25°C in a variable-speed microcentrifuge.
- Use blood stored in EDTA, citrate, or heparin anticoagulants.
- To obtain RNA, use fresh blood only.
- Use blood that has been stored for ≤1 month at +15 to +25°C, +2 to +8°C, or −15 to −25°C. For best results in DNA applications, use fresh blood or blood stored for ≤3 days.

Precautions

Follow all universal safety guidelines governing work with biohazardous materials:
- Wear lab coats, gloves, and safety glasses at all times.
- Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

2.2. Protocols

Lysis of red blood cells from human whole blood

The following procedure was optimized for 500 µl samples of blood. If using <500 µl of blood, see section, **For use with smaller quantities of blood**.

1. For each blood sample to be processed, add 1 ml Red Blood Cell Lysis Buffer to an autoclaved 1.5 ml microfuge tube.

2. To each tube, add 500 µl human whole blood, see section, **General Considerations**.
   – Cap the tube and mix the contents by inversion.
   ⚠ ***Do not vortex.***

3. Place the microfuge tube on a rocking platform or gyratory shaker for 10 minutes at +15 to +25°C.
   – Alternatively, manually invert the sample periodically for 10 minutes.

4. Centrifuge the tube at 500 × *g* for 5 minutes in a microfuge at +15 to +25°C.

5. With a sterile pipette, carefully remove and properly dispose of the clear, red supernatant that is indicative of full red cell lysis.

6 After removal of the supernatant, a white pellet should be visible at the bottom of the tube.

However, if two distinct layers (a cloudy white upper layer containing plasma/leukocytes and a red lower layer containing erythrocytes) are visible in the supernatant, no cell lysis has occurred. If this occurs, do the following:

– Repeat steps 1 to 5 with fresh blood.
– Verify that the Red Blood Cell Lysis Buffer is equilibrated to +15 to +25°C prior to use.
– Invert the sample more frequently if mixing by hand.
– Use a higher ratio, such as 3:1 of Red Blood Cell Lysis Buffer to blood.
– Use a 15 minute incubation in Step 3.

If...	Then...
the sample will be further purified for downstream applications,	disregard Steps 7 to 10.
no further purification will be performed,	continue with Steps 7 to 10 to remove residual RBC material.

7 Add 1 ml Red Blood Cell Lysis Buffer, cap the tube, and mix by flicking the tube until the pellet is resuspended.

⚠ ***Do not vortex.***

8 Centrifuge the tube at 500 × *g* for 3 minutes in a microfuge at +15 to +25°C.

9 With a sterile pipette, carefully remove and properly dispose of the supernatant, particularly the red ring of blood cell debris that forms around the outer surface of the white pellet.

10 Resuspend the white pellet in an appropriate buffer.

For use with smaller quantities of blood

By slightly adjusting the protocol provided in **Lysis of red blood cells from human whole blood**, blood samples of 1 to 500 µl can be processed. Follow the described protocol with these modifications:

Blood volume [µl]	Volume of Red Blood Cell Lysis Buffer in Steps 1 and 7 [µl]
400 – 500	1 ml
300 – <400	800
200 – <300	600
100 – <200	400
25 – <100	200
5 – <25	200
1 – <5	200

3. Supplementary Information

3.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

3.2. Changes to previous version

Layout changes.
Editorial changes.

3.3. Trademarks

All product names and trademarks are the property of their respective owners.

3.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

3.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

3.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

3.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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