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Roche

Red Blood Cell Lysis Buffer

For preferential lysis of red blood cells from human whole blood

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

Version 09
Content version: April 2016

Store at +2 to +8°C

1. What this Product Does

Number of Tests

50 to 500 reactions, depending on sample size (1 to 500 µl)

Contents

100 ml ready-to-use solution.

Storage and Stability

Store the product at at +2 to +8°C until the expiration date printed on the label.

Application

Used for both DNA and RNA isolation, the buffer is 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. This buffer is not intended for use with whole blood from any other species.

2. How to Use this Product

2.1 Before You Begin

General Handling Recommendations

- Warm the buffer to +15 to +25°C prior to use.
  - ⚠ **Do not** use blood that has been frozen and thawed more than three times for any DNA applications.
- Warm blood to +15 to +25°C.
  - ⚠ **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 microfuge.
- 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.

⚠ In addition, be certain to follow all universal safety precautions governing work with biohazardous materials. For example, wear lab coats, gloves, and safety glasses at all times. Also, properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

2.2 Procedure

Lysis of Red Blood Cells from 500 µl Human Whole Blood

The following procedure was optimized for 500 µl samples of blood. If using <500 µl of blood, see "For use with smaller quantities of blood" for necessary modifications to this procedure.

Step	Action
❶	For each blood sample to be processed, add 1 ml Red Blood Cell Lysis Buffer to a sterile 1.5 ml microfuge tube.
❷	To each tube, add 500 µl human whole blood (see "General Handling Recommendations"). Cap the tube, and mix the contents by inversion. Do not vortex.
❸	Place the microfuge tube on a rocking platform or gyratory shaker for 10 min at +15 to +25°C. Alternatively, manually invert the sample periodically for 10 min.
❹	Centrifuge the tube at 500 × *g* for 5 min in a microfuge at +15 to +25°C.
❺	With a sterile pipette, **carefully** remove and properly dispose of the clear, red supernatant that is indicative of complete red cell lysis.
❻	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 so, repeat steps 1 to 5 with fresh blood and also: ▪ verify 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 (*e.g.,* 3:1) of Red Blood Cell Lysis Buffer to blood ▪ use a 15 min incubation in step 3.
❼	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.**
❽	Centrifuge the tube at 500 × *g* for 3 min in a microfuge at +15 to +25°C.
❾	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.
❿	Resuspend the white pellet in an appropriate buffer.

If...	Then...
the sample will be further purified for downstream applications	disregard steps 7 to10
no further purification will be performed	continue with steps 7 to 10 to remove residual RBC material

2.3 For Use with Smaller Quantities of Blood

By slightly adjusting the protocol provided in "For preferential lysis of red blood cells from 500 µl human whole blood," blood samples of 1 to 500 µl can be processed. Follow the procedure described above with these modifications:

For a Blood Volume of	Use the following volume of Red Blood Cell Lysis Buffer in steps 1 and 7 of the procedure
400 to 500 µl	1 ml
300 to <400 µl	800 µl
200 to <300 µl	600 µl
100 to <200 µl	400 µl
25 to <100 µl	200 µl
5 to <25 µl	200 µl
1 to <5 µl	200 µl

3. Additional Information on this Product

How this Product Works

Red Blood Cell Lysis Buffer is designed for the preferential lysis of red blood cells from human whole blood.

Use this buffer on 1 to 500 µl human whole blood (stored one month or less at +15 to +25°C, +2 to +8°C, or −15 to −25°C) to isolate white blood cells that are free of red blood cells. 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. This is important for both DNA and RNA isolation.

Red Blood Cell Lysis Buffer eliminates the need for hazardous organic extractions or chaotropic agents.

The buffer is particularly useful for high-volume research currently requiring Ficoll/Hypaque gradients.

4. Supplementary Information

Changes to Previous Version

- Editorial changes

Text Conventions

To make information consistent and understandable, the following text conventions are used in this document:

Text Convention	Use
Numbered instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed.

Symbols

The following symbol is used in this document to highlight important information:

Symbol	Description
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Ordering Information

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