Red Blood CELL Lysis Buffer (1X)

Filtration, Treatment	Sugar-based, 0.1µm sterile filtered
Product Code	RCL-001-100ML
Shelf Life	18 months from DOM
Storage Temperature	2-8°C
Shipping Temperature	Ambient

QC Specifications

Physical and Chemical Analysis	**Method**	**Specifications**	**Units**
Appearance	Visual	Clear colorless solution	n/a
pH at RT	Electronic pH Meter	7.9 – 8.1	n/a
Endotoxin	LAL Kinetic	<1.0	EU/ml
Sterility			
Aerobic Bacteria	Internally Validated	Not detected	n/a
Anaerobic Bacteria	Internally Validated	Not detected	n/a
Fungi (yeast & Mold)	Internally Validated	Not detected	n/a

Product Description and Applications

Our Red Blood Cell Lysis Buffer is a sugar-based buffer whose formulation has been optimized for the preferential lysis of red blood cells from human whole blood, while having minimal effect of leukocytes, including lymphocytes. As Sugars at high temperature can cause caramelization (browning), which degrades the sugars, therefore autoclaving is not the best option for sterilization of such products. Therefore, our RCL products are sterilized using pharmaceutical-grade filtration.

Using our RCL buffers for the lysis of erythrocytes from human whole blood, yields intact white blood cells free of red blood cells for further applications, particularly for DNA and RNA isolation of white blood cells. Most blood cells are red blood cells, which lack nuclei and possess no DNA. Therefore, the lysis using proper buffer followed by centrifugation steps eliminate the red cells and concentrates the nucleated white blood cells.

Our RCL Buffers eliminate the need for hazardous organic extractions or chaotropic agents.

Instruction for Use

We highly recommend using fresh blood or blood not older than one week. Please do not use blood older than one month or kept under several cycles of thawing-refreezing. For RNA extraction use only fresh blood.

1. Keep both RCL buffer and whole blood sample at room temperature for 15 to 25°C for minimum 10 minutes before starting.
2. If you use the RCL 5X dilute RCL 5X to 1X using sterile Purified Water (Product code: **BWL-001**). If RNA extraction is intended, we highly recommend using our RNase-free Molecular Biology grade Water (Product code: **BWL-002**).
3. Prepare 500µl samples of blood in 1.5 ml sterile microfuge tube.
4. Add 1ml of diluted RCL buffer to each tube. Cap the tube and mix the contents by inversion**. Do not vortex!**
5. Place the tubes on a rocking platform or gyratory shaker and shake gently for 5-10 minutes at room temperature.
6. Centrifuge the tubes at 500 × *g* for 5 minutes at room temperature.
7. Discard red clear supernatant 8indicative of full red cell lysis).
8. Check the color of the pellet at the bottom of the tube. If a monolayer white pellet of leukocytes is fully visible go the step 9. If a double-layer cloudy pellet including the upper white cells and lower red cells is visible, repeat steps 4-8 one-three times. Note, breakdown the pellet by vortexing and rinses it well in red blood cell lysis buffer in order to clean the white blood cells from the residual of hemoglobin. To reduce the required number of repeating cycles, higher ratio of RCL buffer to the blood sample (e.g. 3:1 V/V) can be used from the beginning.
9. 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!**
10. Centrifuge the tube at 500 × *g* for 3 minutes at room temperature.
11. 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.
12. Resuspend the white pellet in an appropriate buffer

Precautions and Disclaimer

This product is intended to use for life science research use only. Not for use in diagnostic procedures.

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.
- See the product Material Safety Data Sheet for more information.

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