ab204733
Red Blood Cell (RBC) Lysis Buffer

Instructions for Use

For the lysis of red blood cells.

This product is for research use only and is not intended for diagnostic use.
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1. **BACKGROUND**

Red Blood Cell (RBC) Lysis Buffer (ab204733) provides a quick and efficient method of lysing red blood cells. Red Blood Cell (RBC) Lysis Buffer uses ammonium chloride method to lyse red blood cells without affecting leukocytes, normal tissue, or tumor cells.

Human whole blood is composed of 45% red blood cells. Without the removal of red blood cells, it is difficult to analyze the phenotype and function of leukocytes in whole blood. Haemoglobin and other red blood cell contents can also interfere with several chemical assays.
2. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

3. **STORAGE AND STABILITY**

Store product at room temperature upon receipt. Product has a storage time of 1 year from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

4. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Red Blood Cell (RBC) Lysis Buffer</td>
<td>100 mL</td>
<td>RT</td>
<td>RT</td>
</tr>
</tbody>
</table>

6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH$_2$O)
- Microcentrifuge
- Pipettes and pipette tips

7. **TECHNICAL HINTS**

- Make sure all buffers and solutions are at room temperature before starting the experiment.
8. **REAGENT PREPARATION**

8.1. **Red Blood Cell (RBC) Lysis Buffer:**

To prepare the 1X Red Blood Cell (RBC) Lysis Buffer, dilute 10 mL of Red Blood Cell (RBC) Lysis Buffer with 90 mL of dH2O.

If cells present in the sample are going to be cultured after RBC lysis, use sterile water.
9. **ASSAY PROCEDURE**

- Always take precautions when handling human samples.

9.1. **RBC lysis from whole blood:**

9.1.1 Add 20 volumes of 1X RBC Lysis buffer to 1 volume of whole blood.

9.1.2 Incubate for 5 -10 minutes at room temperature.

9.1.3 Centrifuge at 400 x g for 5 minutes. Remove the supernatant carefully.

9.1.4 Re-suspend the cell pellet in appropriate buffer. Cells are ready for further analysis.

**NOTE:** Repeat the above lysis protocol if necessary needed to remove all traces of red blood cells

9.1. **RBC lysis from tissue or solid tumor samples:**

9.2.1 Dissociate tissue or solid tumor samples into single cells.

9.2.2 Centrifuge cells at 400 x g for 5 minutes at room temperature. Remove the supernatant carefully.

9.2.3 Resuspend cell pellet in 1X RBC Lysis Buffer.

- Sample with < 1 x 10^8 cells = use 5 mL 1X RBC Lysis buffer
- Samples with > 1 x 10^8 cells = use 10 mL 1X RBC Lysis buffer

9.2.4 Incubate for 5-10 minutes at room temperature.

9.2.5 Centrifuge at 400 x g for 5 minutes at room temperature. Remove the supernatant carefully.

9.2.6 Re-suspend the cell pellet in appropriate buffer. Cells are ready for further analysis.
10. **TYPICAL DATA**

![Graph showing absorbance (OD 550 nm) for PBS, Whole Blood, and RBC Lysis Buffer]

**Figure 1.** Human whole blood (100 μL) was added to 1X Red Blood Cell (RBC) Lysis Buffer (2 mL) and incubated for 8 minutes at room temperature. After incubation, cells were centrifuged and resuspended in 1 mL of PBS. Absorbance (550 nm) was measured by Spectrophotometer. Removal of red blood cells results in reduced absorbance in RBC Lysis Buffer compared to whole blood sample.
11. FAQ
12. **NOTES**
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp

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