Miltenyi Biotec

Red Blood Cell Lysis Solution (10x)

For *in vitro* diagnostic use

IVD CE

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1. General information

Intended use
Red Blood Cell Lysis Solution (10×) is intended for lysing red blood cells in combination with immunofluorescence labeling of human peripheral blood cells with antibody-fluorochrome reagents for *in vitro* diagnostic use in flow cytometry.

Reagents and contents

Product	Σ 250	Volume	REF
Red Blood Cell Lysis Solution (10×)	for 250 tests	50 mL	170-080-033

2. Technical data and background information

Components	50mL Red Blood Cell Lysis Solution (10×)
Product formulation	Red Blood Cell Lysis Solution (10×) is a ammonium chloride (NH_4Cl) based solution, pH 7.5
+2°C +8°C	Store at +2 °C to +8 °C. Do not freeze.
	Store protected from light.
	The use-by date is indicated on the vial label.
	For in-use stability at +2 °C to +8 °C storage temperature refer to the use-by date indicated on the vial label. Do not use reagent after the use-by date.

Background information
Red Blood Cell Lysis Solution (10×) has been developed for the lysis of red blood cells to ensure optimal lysis of erythrocytes with minimal effect on all cell types obtained from peripheral blood. A washing step after erythrocyte lysis is optional, depending on the application. The solution is suitable for lysis of erythrocytes in single-cell suspensions of human origin.

3. Warnings and precautions

- ▲ Analysis results obtained by use of the reagents shall never be the sole basis for classification of disease states.
- ▲ Interpretation of results is under the full responsibility of the user.
- ▲ For all handling, consideration of good laboratory practice (GLP) regulations is recommended.
- ▲ Use of the reagents is restricted to trained and qualified personnel only.
- ▲ All biological specimens and all materials that come into contact with blood and blood products must be treated as infectious material. Regulations for the treatment and disposal of infectious material must be followed.
- ▲ The reagent contains ammonium chloride (NH_4Cl). Although this mixture is not classified as dangerous at product concentration, it is recommended to avoid contact with eyes. For detailed information refer to the safety data sheet which is available on request.
- ▲ For material required but not provided the manufacturers recommendations and safety regulations must be followed.
- ▲ Reagents should not be used if signs of leakage are observed. Use undamaged and sealed vials only.

Directions of the package insert must be followed to obtain accurate and reproducible results.

4. Application

Efficient detection of leucocytes in flow cytometric analyses depends on the elimination of interfering cells. Red Blood Cell Lysis Solution (10×) can be used for lysing erythrocytes in combination with immunofluorescence staining of human peripheral blood cells with antibodies prior to immunophenotyping by flow cytometry.

5. Materials required but not provided

- For bulk lysis: 50 mL polypropylene conical tubes 30×115 mm or 15 mL high clarity polypropylene conical tubes 17×120 mm, dependent on volume
- Disposable capped polystyrene tubes, 12×75 mm
- PEB Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting e.g. MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (+2 °C to +8 °C).
- Double-distilled water
- Micropipettes with disposable tips: variable micropipettes with volume ranges of 10–100 µL and 100–1000 µL
- Low speed centrifuge: minimum speed 300×g, with 12×75 mm tube carriers
- Vortex mixer
- Flow cytometer with appropriate laser and filter settings
- Fluorochrome-conjugated antibodies directed to leukocyte antigens

6. Protocol

Principle of method:
A sample of interest, e.g. whole blood, gets incubated with fluorochrome-conjugated antibodies, which bind specifically to their antigens expressed by leukocyte subsets. After that, erythrocytes, which could interfere with the detection of the target cell population, are eliminated by using the Red Blood Cell Lysis Solution. The deletion of erythrocytes can also be done prior to the immunofluorescence labeling of cells during a bulk lysis. Analysis of the sample is then performed in a flow cytometer at a single-cell level.

Important notes:
Under some conditions red blood cells may not lyse within 10 minutes. In this case extend lysis time to 20 minutes before centrifugation of samples.

Exposure of reagents to temperatures below +2 °C and above +8 °C and to light should be minimized during handling.

Sample requirements
- Reagents can be used for determination of antigen-positive cells in whole blood samples by flow cytometry. Each cell source can have different storage conditions and limitations that should be considered prior to collection and analysis. For collection of patient samples European and national legislation must be followed.
- Whole Blood samples should be stained within 24 hours.
- Viability of the cells should be assessed and use of samples with at least 80% viable cells is suggested in order to minimize the risk of erroneous results.
- Cell count of white blood cells should not exceed 5×10^7 cells/mL.

  Note: If necessary dilute cell sample with PEB buffer.

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Protocol

Bulk lysis procedure

1. Dilute 10× Red Blood Cell Lysis Solution 1:10 with double- distilled water (ddH_2O). For example, dilute 2 mL of 10× Red Blood Cell Lysis Solution with 18 mL of ddH_2O.

   Note: Do not dilute with deionized water. Store prepared 1× Red Blood Cell Lysis Solution at room temperature. Discard unused solution at the end of the day.

2. Add an appropriate amount of whole blood to a suitable tube (e.g. 50 ml or 15 ml capacity).

3. Add 1× Red Blood Cell Lysis Solution in 20-fold excess to the whole blood. For example, add 20 mL of 1x Red Blood Cell Lysis Solution to 1 mL of whole blood.

4. Immediately vortex thoroughly for 3 seconds and incubate for 10 to 20 minutes at room temperature in the dark.

5. Centrifuge at 300×g for 10 minutes. Remove supernatant.

6. Optional: Wash cells by adding the 20x volume of buffer of your initial cell sample volume.

   Centrifuge at 300×g for 10 minutes. Remove supernatant.

7. Resuspend cell pellet in a suitable amount of buffer and proceed to immunofluorescence staining.

Stain-Lyse-Wash Procedure

1. Dilute 10x Red Blood Cell Lysis Solution 1:10 with double-distilled water (ddH_2O). For example, dilute 1 mL of 10× Red Blood Cell Lysis Solution with 9 mL of ddH_2O.

   Note: Do not dilute with deionized water. Store prepared 1× Red Blood Cell Lysis Solution at room temperature. Discard unused solution at the end of the day.

2. Add 100 µL whole blood to a 12x75 mm tube.

3. Add the recommended amount of fluorochrome-conjugated antibody.

4. Mix well and incubate for 15 minutes in the dark at room temperature (+20 °C to +25 °C).

   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

5. Add 2 mL of 1× Red Blood Cell Lysis Solution to each tube. Immediately vortex thoroughly for 3 seconds and incubate for 10 to 20 minutes at room temperature in the dark.

6. Centrifuge at 300×g for 10 minutes. Remove supernatant.

7. Wash cells by adding 1–2 mL of buffer, centrifuge at 300×g for 10 minutes. Remove supernatant.

8. Resuspend cell pellet in a suitable amount of buffer and proceed to flow cytometric analysis. Store samples at +2 °C to +8 °C until analysis.

   Note: Minimize exposure of samples to light.

Quality control

It is recommended to run regularly a control sample from a normal adult specimen or commercially available whole blood control as a quality control of the system.

7. Performance characteristics

Precision

Red Blood Cell Lysis Solution (10×) was tested by flow cytometry using a bulk lysis and a stain- lyse-wash protocol on whole blood from healthy donors, respectively. Reproducibility was assessed by measuring the frequency of erythrocytes and leukocytes in replicate measurements performed by different operators using the same set of different donor samples. Precision was inferred from calculating the mean, standard deviation and coefficient of variation of the frequency of red and white blood cells. All values were within the acceptance criterion.

Analytical specificity

Ammonium chloride-based lysis solution is a well-documented reagent for the elimination of red blood cells in human blood samples. The results have been published and are referenced in Andrey V. Chernyshev *et. al*, Journal of Theoretical Biology 251 (2008) 93–107 and EuroFlow Standard Operating Protocol for Bulk Lysis for MRD Panels, Version 1.0, March 20, 2014 and Gratama JW *et.al*, J Immunol. Methods 239: 13-23 (2000).

8. Limitations

Reagent data performance was collected typically with EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

9. References

1. De Paoli, P. *et.al*, Enumeration of human lymphocyte subsets by monoclonal antibodies and flow cytometry: a comparative study using whole blood or mononuclear cells separated by density gradient centrifugation. J Immunol Methods 72:349-353, 1984
2. Ashmore, L.M. *et.al*, Lymphocyte subset analysis by flow cytometry. Comparison of three different staining techniques and effects of blood storage. J Immunol Methods 118:209-215, 1989
3. Renzi, P. *et.al,* Analysis of T cell subsets in normal adults: comparison of whole blood lysis technique to Ficoll-Hypaque separation by flow cytometry. J Immunol Methods 98:53-56, 1987
4. Romeu, M.A. *et.al*, Lymphocyte immunophenotyping by flow cytometry in normal adults: comparison of fresh whole blood lysis technique, Ficoll-Paque separation and cryopreservation. J Immunol Methods 154:7-10, 1992
5. Jackson, A. *et.al*, Basic phenotyping of lymphocytes: selection and testing of reagents and interpretation of data. Clin Immunol Newslett 10(4):49-55;1990
6. Kidd, P.G. *et.al*, Report of the workshop on the evaluation of T-cell subsets during HIV infection and AIDS. Clin Immunol Immunopathol 52:3-9; 1989
7. Landay, A.L. *et.al*, Procedural guidelines for performing immunophenotyping by flow cytometry. Clin Immunol Immunopath 52:48-60; 1989
8. Chernyshev A.V. *et.al*, "Erythrocyte lysis in isotonic solution of ammonium chloride: Theoretical modeling and experimental verification", Journal of Theoretical Biology 251 (2008) 93–107
9. EuroFlow Standard Operating Protocol for Bulk Lysis for MRD Panels, Version 1.0, March 20, 2014
10. Gratama, J.W. *et.al*, Loss of CD34+ hematopoietic progenitor cells due to washing can be reduced by the use of fixative-free erytrocyte lysing reagents. J Immunol. Methods 239: 13-23 (2000).

10. Glossary of symbols

Manufacturer	In-use stability
REF Order number	+2°C +8°C Temperature limit
P/N Part number	Protect from sunlight
LOT Batch code	European conformity approval
IVD *In vitro* diagnostic medical device	Phone
Use-by date	Website
Consult instruction for use	