Technical Data Sheet

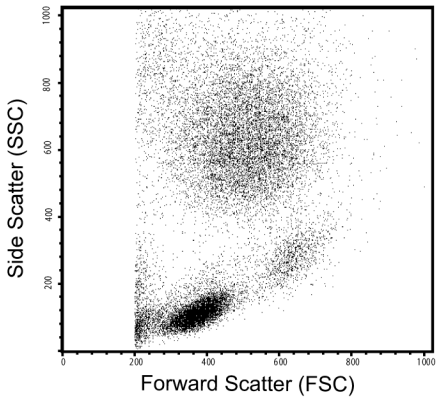
Lysing Buffer

Product Information

Material Number: **555899**
Size: 100 ml

Description

BD Pharm Lyse™ lysing solution is a buffered, concentrated (10X) ammonium chloride-based lysing reagent. When diluted to a 1X concentration and used as recommended, BD Pharm Lyse™ lyses red blood cells following monoclonal antibody staining. The lysing solution results in good light scatter separation of lymphocytes and red blood cell debris when analyzed by flow cytometry. BD Pharm Lyse™ does not contain a fixative agent, so leukocytes remain viable after red blood cell lysis.



Scatter profile of human whole blood treated with BD Pharm Lyse™.

Preparation and Storage

Store undiluted at 4°C.
1X diluted solutions may be stored at 4° C for up to 30 days.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

Preparation of 1X lysing solution: Dilute the 10X concentrate 1:10 with distilled water. The pH of the 1X solution should fall within the range of pH 7.1-7.4. Adjust the pH if necessary. Warm the 1X solution to room temperature prior to use. 100 ml of 10X concentrate will yield a quantity of 1X solution that is sufficient to lyse 500 samples.

Lysing procedure:

Note: The following procedure is only applied to human whole blood red blood cell lysis. Since applications vary, for other samples such as mouse spleen red blood cell lysis (lysing incubation time up to 3 minutes at 37°C), bone marrow red blood cell lysis, each investigator should optimize the condition to obtain optimal results.

1. Add 2.0 ml of 1X lysing solution to each tube containing up to 200 µl of a whole blood plus monoclonal antibody mixture.
2. Gently vortex each tube immediately after adding the lysing solution.
3. Incubate at room temperature, protected from light, for 15 minutes.
4. Centrifuge 200 X g for 5 minutes.
5. Carefully aspirate supernatent, without disturbing pellet.
6. Add 2.0 ml 1X PBS containing 1% heat-inactivated fetal bovine serum and 0.1% sodium azide (PBS-FBS).
7. Centrifuge at 200 X g for 5 minutes.
8. Carefully aspirate supernatent, without disturbing pellet.
9. Resuspend pellet in 0.5 ml PBS-FBS or a fixative such as 2% formaldehyde for flow cytometric analysis.

Troubleshooting: Incomplete lysis may occur for several reasons: (1) The age of the specimen may affect red cell lysis, (2) The 1X lysing solution may not have been warmed to room temperature, or (3) After adding the lysing solution, the samples may not have been vortexed

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sufficiently. If incomplete lysis occurs (recognized by the presence of an excessive amount of visible red blood cells in the final, washed cell suspension), repeat lysing procedure. However, lysing more than two times is not recommended.

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Muirhead KA, Wallace PK, Schmitt TC, Frescatore RL, Franco JA, Horan PK. Methodological considerations for implementation of lymphocyte subset analysis in a clinical reference laboratory. *Ann N Y Acad Sci.* 1986; 468:113-127. (Methodology: Flow cytometry)