

OptiLyse® B

Lysing Solution

250 Tests PN IM1400

For Research Use Only.

Not for use in diagnostic procedures.

1. INTENDED USE

OptiLyse B Lysing Solution is an erythrolytic reagent intended for the lysis of red blood cells in the preparation of biological samples for flow cytometry analysis after staining of leucocytes with fluorescent antibodies.

Use of OptiLyse B is limited to samples for analysis using BD Biosciences flow cytometers and is compatible with so-called «no wash» staining and lysis procedures as long as duly calibrated fluorescent antibodies are used for this purpose.

2. SUMMARY AND EXPLANATION

In flow cytometry it is imperative that leucocytes be analyzed free from interference by erythrocytes. In the past, this has been accomplished by density gradient separation or by red blood cell lysis with several washing steps. However, it has been clearly demonstrated that centrifugal washing may alter the remaining cellular distribution (1).

The separation of debris and leucocytes is easily accomplished when OptiLyse B Lysing Solution is used in a method which involves no centrifugation or washing steps. OptiLyse B Lysing Solution is a premixed protective erythrolytic reagent formulated for use with BD Biosciences flow cytometers.

Following immunostaining, the reagent lyses erythrocytes and fixes leucocytes resulting in a leucocyte suspension substantially free of red blood cells and suitable for flow cytometry.

3. APPLICATION

Immunophenotypic analysis of peripheral blood leucocytes is facilitated by use of an erythrolytic reagent. Immunostaining followed by whole blood lysis is the preferred method for providing flow cytometric results.

4. PRINCIPLE OF THE TEST

The biological sample containing red blood cells for lysis is incubated in the presence of the OptiLyse B solution, which results in the lysis of red blood cells accompanied by the fixation of leucocytes.

A specific staining of leucocytes is first achieved by incubating the sample with the antibody or antibodies selected. The red cells are then removed by lysis and the leucocytes, which are fixed during this stage, are analyzed by flow cytometry.

5. REAGENT

OptiLyse B Lysing Solution, PN IM1400, 250 tests (25 mL), Ready for use.

OptiLyse B Lysing Solution is a buffered solution containing 3.4% formaldehyde. This reagent contains no azide and is not light-sensitive.

6. MATERIALS REQUIRED BUT NOT PROVIDED

1. Conjugated Antibodies calibrated for "no-wash" procedures (e.g., OptiClone® Monoclonal Antibodies, Beckman Coulter).
2. Deionized water.
3. Whole blood collection tubes with anticoagulant (EDTA, ACD, Heparin).
4. 12 x 75 mm test tubes.
5. Pipettors and pipet tips capable of delivering 100 µL and 1000 µL.
6. Timer.
7. Vortex mixer.
8. Flow cytometer.

7. STATEMENT OF WARNINGS

1. This reagent contains formaldehyde. Formaldehyde is toxic and allergenic. It is thought to be a carcinogenic agent. Never pipette by mouth and avoid all contact with the skin, mucosae, eyes and clothing. If skin or eye contact occurs, wash excessively with water.
2. All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
3. Never pipet by mouth and avoid contact of specimens, samples, or reagents with skin, mucous membranes, and eyes.
4. Do not use the reagent beyond expiry date.
5. Incubation times or temperatures other than those specified may give erroneous results.
6. Avoid microbial contamination of the reagent or erroneous results may occur.
7. Do not store in the refrigerator, do not freeze.
8. Do not expose reagents to strong light during storage or incubation.
9. Use general good laboratory practices when handling this reagent.

8. STORAGE CONDITION AND STABILITY

Store OptiLyse B Lysing Solution at 18 – 25°C.

OptiLyse B Lysing Solution is stable at 18 – 25°C until the expiration date printed on the bottle.

9. REAGENT PREPARATION

No preparation is necessary. OptiLyse B Lysing Solution is used directly from the bottle with no further dilution or preparation.

10. BLOOD SAMPLE COLLECTION

Venous blood samples must be taken using sterile tubes. A salt of EDTA is recommended as anticoagulant, however ACD and heparin may also be used.

Store anticoagulated whole blood at 18 – 25°C. Specimens should be processed within 6 hours when possible. If not possible, the laboratory should verify that holding time and conditions maintain specimen integrity comparable to fresh specimens.

The sample should be homogenized by gentle agitation prior to taking the test sample.

The samples must be analyzed within 24 hours of venipuncture.

11. SAMPLE PREPARATION

Determine the absolute white blood cell count. In case of white cell count > 10 x 10⁹ cells/L, dilute blood in phosphate-buffered saline to obtain approximately 5 x 10⁹ cells/L (5 x 10³ cells/µL).

12. PROCEDURE

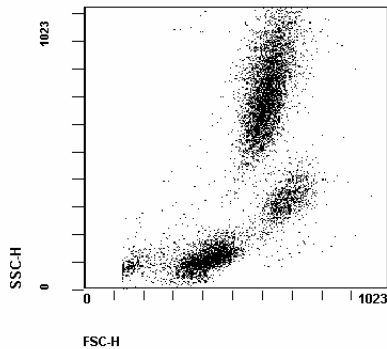
1. Follow instructions for use of conjugated antibodies or isotopic controls for each specimen.
2. Add 100 µL of OptiLyse B Lysing Solution to one tube at a time. **Each addition should be followed immediately by a brief vortex mixing before proceeding to the next step.** Tightly seal OptiLyse B Lysing Solution after use.
3. Allow all tubes to incubate at room temperature (18 – 25°C) for 10 minutes.
4. Add 1000 µL of deionized water followed by brief vortex mixing.
5. After 10 minutes, analyze cell preparations by flow cytometry. Preparations may be stored for 24 hours at 2 – 8°C without adversely affecting results.

13. FLOW CYTOMETRIC ANALYSIS

Analyze stained cells according to the instrument manufacturer's guidelines. Lymphocytes should be identified on the basis of dual parameter forward angle and perpendicular angle light scatter histograms. The determination of positivity / negativity for immunostained cells should be based on the non-specific fluorescence of the isotopic control-stained cells.

14. EXAMPLE OF DATA

The dual-parameter light scatter histogram below (FS versus SS) illustrates the expected leucocyte spatial distribution of a normal K₃EDTA anticoagulated blood sample lysed with OptiLyse B Lysing Solution (PN IM1400) Acquisition is with with a BD FACSCalibur™ flow cytometer and analysis is with EXPO™32 analysis software.



15. LIMITATIONS

1. OptiLyse Lysing Solution and Antibodies should be used in conjunction with an ongoing daily laboratory quality control program to monitor instrument performance and determine reference ranges for immunostained cells under the conditions used. This should be monitored daily by a comprehensive program of standards and controls. Participation in interlaboratory proficiency testing programs is encouraged.

2. Appearance of erythrocytes, excessive debris, or monocytes in the lymphocyte light scatter area should be addressed. Verify the lymphocyte gate by determining the recovery of lymphocyte within the gate and the lymphocyte purity of the gate.
3. Specimens with nucleated red blood cells may show incomplete lysis of red blood cells.
4. In certain blood samples red blood cell lysis may be slow, ineffective or even impossible. If this is the case, we recommend separating mononucleated cells in a Ficoll-Hypaque density gradient before labelling.
5. Results obtained with flow cytometry may be erroneous if the instrument is misaligned, compensation spillovers are not correctly compensated or the gates are improperly set.
6. OptiLyse B Lysing Solution should be used with BD Biosciences flow cytometers.
7. The stability of blood samples is quite variable. Start the assay within 6 hours of venipuncture for optimal lysis results on whole blood samples. Unstained, anticoagulated blood specimens should remain at 18 – 25°C until processing.
8. Do not refrigerate blood, as this may result in erroneous subset percentages.

16. REFERENCES

1. Caldwell, C.W., and Taylor, H.M.: A rapid, no-wash technique for immuno-phenotypic analysis by flow cytometry. Am J Clin Pathol 86:600-607, 1986.

17. TRADEMARKS

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Manufactured by:
 Immunotech SAS, a Beckman Coulter Company
 130, avenue de Lattre de Tassigny, B.P. 177
 13276 Marseille Cedex 9, France.

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