BD FACS Lysing Solution

10X Concentrate

100 mL— **Catalog No. 349202**

1/2010 23-1358-06

IVD

1. INTENDED USE

BD FACS™ Lysing Solution* is intended for lysing red blood cells following direct immunofluorescence staining of human peripheral blood cells with monoclonal antibodies prior to flow cytometric analysis.

FACS Lysing Solution is appropriate for use with reagents such as BD TriTEST™ or Simultest™ reagents and a suitably equipped flow cytometer. It may be used in both lyse/wash and lyse/no-wash procedures.

2. SUMMARY AND EXPLANATION

Efficient detection of lymphocytes in peripheral blood depends on the elimination of interfering cells. Whole blood lysis has been shown to be as effective as density gradient centrifugation in the preparation of peripheral blood mononuclear cells (PBMCs) for lymphocyte subset analysis.[1-4] In clinical laboratories, whole blood lysis methods have essentially replaced Ficoll-Paque density gradient separation because of shorter sample preparation time and less handling of whole blood.[5] Studies have also shown that the lysed whole blood method is less likely to show loss of lymphocyte subsets and may help improve assay reproducibility when compared to earlier methods.[5-7]

3. PRINCIPLES OF THE PROCEDURE

When whole blood is added to the monoclonal antibody reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leucocyte surface antigens. The stained samples are then treated with FACS Lysing Solution which lyses erythrocytes under gentle hypotonic conditions while preserving the leucocytes.

*US Patent Nos. 4,654,312; 4,902,613; and 5,098,849

4. REAGENT

Reagent Provided

FACS Lysing Solution, 10X concentrate, is provided as 100 mL of a proprietary buffered solution containing <15% formaldehyde and <50% diethylene glycol. This quantity is sufficient for 2,000 tests when used in BD lyse/no-wash procedures (eg, TriTEST), and for 500 tests when used in lyse/wash procedures (eg, Simultest).

Precautions

1. For in vitro diagnostic use.
2. **WARNING** : Reagent contains diethylene glycol and formaldehyde. Formaldehyde is harmful by inhalation, in contact with skin, and if swallowed. It is irritating to eyes and skin. Exposure can cause cancer. Possible risks of irreversible effects. May cause sensitization by skin contact. Keep locked up and out of the reach of children. Keep away from food, drink, and animal feedingstuff. Wear suitable protective clothing and gloves. Even small amounts of diethylene glycol can be fatal. If swallowed, seek medical advice immediately and show this container or label. Dispose of according to federal, state, and local regulations.
3. All patient specimens and materials with which they come into contact are considered biohazards and should be handled as if capable of transmitting infection.[8,9]

   WARNING : Follow proper precautions in accordance with federal, state, and local regulations when disposing of all materials. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.

Dilution Instructions

Dilute the 10X concentrate 1:10 with room temperature (20° to 25°C), deionized water. The prepared solution is stable for 1 month when stored in a glass container at room temperature.

Storage and Handling

FACS Lysing Solution (10X) is stable for the period shown on the bottle label when stored as directed. Do not use this reagent if discoloration occurs or a precipitate forms.

5. INSTRUMENT

FACS Lysing Solution is designed for flow cytometers equipped with appropriate computer hardware and software. The flow cytometer must be equipped to detect forward scatter (FSC) and side scatter (SSC). BD recommends a FACSCalibur™, FACSort™, or FACScan™ flow cytometer; however, results may be achieved using other platforms. Refer to the appropriate reagent package insert for specific instrument limitations.

6. SPECIMEN COLLECTION AND PREPARATION

Collect blood aseptically by venipuncture[10,11] into a sterile K_3 EDTA (lavender top) VACUTAINER® blood collection tube. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected. Store anticoagulated blood at room temperature (20° to 25°C) until ready for staining and lysing. Refer to the appropriate reagent package insert for storage restrictions prior to staining. See Section 9, Limitations, for possible interfering conditions.

7. PROCEDURE

Reagent Provided

FACS Lysing Solution, 10X concentrate (BD Catalog No. 349202).

Reagents and Materials Required but Not Provided

1. 1X FACS Lysing Solution, diluted as indicated in Section 4, Reagent: Dilution Instructions.
2. K_3 EDTA VACUTAINER blood collection tubes (BD Catalog No. 356457) or equivalent.
3. Disposable 12 x 75-mm test tubes (Falcon™ capped polystyrene test tubes, BD Catalog No. 352058; or equivalent).
4. BD monoclonal antibodies to human leucocyte antigens (eg, TriTEST or Simultest reagents).
5. Vortex mixer.
6. Micropipettor with tips (BD Electronic Pipette, BD Catalog No. 343246 or equivalent).

Other materials can be required; refer to the appropriate reagent package insert for more information.

Preparing Samples

Stain whole blood samples following specific instructions in the appropriate reagent package insert. Lyse red blood cells as directed using diluted (1X) FACS Lysing Solution. Use care to protect the tubes from direct light. Perform the procedure at room temperature (20° to 25°C).

1. For each sample, combine appropriate amounts of fluorochrome-conjugated monoclonal antibody reagent and blood per tube as directed in the specific package insert.
2. Incubate the tubes as specified.
3. Add an appropriate volume of 1X FACS Lysing Solution to the tubes as directed. Vortex thoroughly.
4. Continue as directed in the specific package insert until the cells are ready to be acquired on the flow cytometer. Cap tubes and store at 2° to 8° in the dark until flow cytometric analysis. Analyze the stained cells within the time limit specified in the appropriate package insert. Vortex the cells thoroughly at low speed to reduce aggregation before acquiring.

8. RESULTS

The following representative data were obtained with peripheral blood samples treated with FACS Lysing Solution on a FACScan flow cytometer. The whole blood was stained with TriTEST CD3/CD4/CD45 reagent (Figure 1) or Simultest CD3/CD4 reagent (Figure 2).

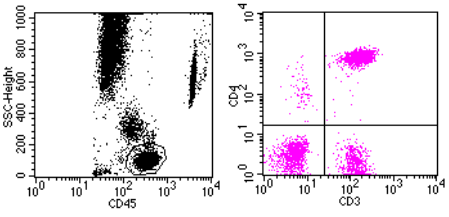

Figure 1. CD45 vs SSC dot plot; CD3 vs CD4 dot plot obtained with TriTEST reagent.

9. LIMITATIONS

1. Laboratories must establish their own normal reference ranges for each reagent parameter that can be affected by sex of patient, age of patient,

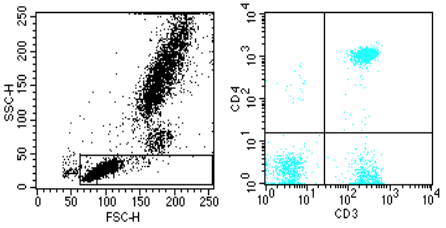

Figure 2. FSC vs SSC dot plot; CD3 vs CD4 dot plot obtained with Simultest reagent.

and preparative technique. Race of patient may also have an affect,[12] although sufficient data are not available to establish this. Age, sex, clinical characteristics and race of subjects should be known when a reference range is determined.[13] Reference ranges provided are for information only.

2. FACS Lysing Solution is specifically formulated for use with BD FACS brand flow cytometers.
3. EDTA is the anticoagulant of choice. BD has limited information concerning use of other anticoagulants such as heparin.
4. Retain samples in VACUTAINER blood collection tubes at room temperature (20° to 25°C) prior to staining and lysing. Refer to the reagent package insert for maximum storage times after collection.
5. Samples with nucleated red blood cells may show incomplete lysis of red blood cells because FACS Lysing Solution does not lyse nucleated erythrocytes. This may also occur when assaying blood samples from patients with certain hematologic disorders in which red cells are difficult to lyse, as in myelofibrosis, sickle-cell anemia, thalassemia, and spherocytosis.[7,8]
6. When using monoclonal reagents which react with serum immunoglobulins, blood samples should be washed with 1X phosphate-buffered saline or physiological saline prior to staining and lysing.[14]
7. A monoclonal reagent against a cell surface antigen or receptor which is: a) shed into plasma (eg, IL-2 receptor) or b) occupied by plasma components (eg, complement receptors) can have reduced staining intensity when analyzed with the lysed whole blood methodology.

10. EXPECTED VALUES

Normal subjects were studied at three clinical sites to establish reference ranges for the $CD3^+$ and $CD3^+CD4^+$ lymphocyte subsets. The reference ranges for the parameters studied are presented in the table below. The ranges obtained were tested for differences by site, sex, and age of subject. If the comparison indicated a significant difference, separate ranges were given.

Lymphocyte subset	Sex	Age	n	Mean	95% range
% $CD3^+$	both	18-70	160	72	59-85
% $CD3^+CD4^+$	male female	18-70 18-70	84 75	43 46	29-57 31-60

Adult reference ranges should not be used with pediatric blood samples (ages neonate to 13 years). Refer to the first limitation for more information about reference ranges.

11. PERFORMANCE CHARACTERISTICS

Precision

For the TriTEST (CD3/CD4/CD45) reagent, specimens from 17 normal and 61 abnormal donors were obtained at two clinical sites. Three aliquots of each specimen were stained, lysed, and run on FACScan flow cytometers.

Subjects	Lymphocyte Subset	n	Mean	SD	DF[a]
normal	%$CD3^+$	17	72	0.72	34
	% $CD3^+CD4^+$	17	42	0.79	34
abnormal	%$CD3^+$	61	77	0.88	122
	% $CD3^+CD4^+$	61	19	0.61	122

a. DF = degrees of freedom: the number of observations (3) minus the number of means (1) multiplied by the number of subjects (n).

For the Simultest (CD3/CD4) reagent, within-specimen reproducibility was assessed at one clinical site. Determinations were made on blood specimens from six normal and four patient subjects (HIV and renal transplant). Two aliquots from the same blood sample were prepared with the Simultest IMK

Lymphocyte reagent panel and each aliquot was run twice on the same FACScan flow cytometer.

Subjects	Lymphocyte subset	n	Mean[a]	SD	DF[b]
normal	%$CD3^+$	6	73	0.85	12
	% $CD3^+CD4^+$	6	46	1.08	12
abnormal	%$CD3^+$	4	83	0.77	8
	% $CD3^+CD4^+$	4	32	1.06	8

a. mean is the pooled mean; ie, the mean of the individual means.
b. DF = degrees of freedom: the number of observations (4) minus the number of means (2) multiplied by the number of subjects (n).

White Cell Recovery

Five blood samples were treated with FACS Lysing Solution, washed, and analyzed for white cell recovery using the ELT-1500™ Clinical Hematology Analyzer. Compared to the total white blood cell count, white cell recovery with the lysing solution averaged 92%.

Red Blood Cell Lysis

Five blood samples were treated with FACS Lysing Solution, washed, and analyzed for residual red blood cells using the ELT-1500 Clinical Hematology Analyzer. No red blood cells were detected.

REFERENCES

1. de Paoli P, Reitano M, Battistin S, Castiglia C, Santini G. 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.* 1984;72:349-353.
2. Ashmore LM, Shopp GM, Edwards BS. Lymphocyte subset analysis by flow cytometry. Comparison of three different staining techniques and effects of blood storage. *J Immunol Methods.* 1989;118:209-215.
3. Renzi P, Ginns LC. Analysis of T cell subsets in normal adults: comparison of whole blood lysis technique to Ficoll-Hypaque separation by flow cytometry. *J Immunol Methods.* 1987;98:53-56.
4. Romeu MA, Mestre M, González L, 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.* 1992;154:7-10.
5. Jackson A. Basic phenotyping of lymphocytes: selection and testing of reagents and interpretation of data. *Clin Immunol Newslett.* 1990;10(4):49-55.
6. Kidd PG, Vogt RF, Jr. Report of the workshop on the evaluation of T-cell subsets during HIV infection and AIDS. *Clin Immunol Immunopathol.* 1989;52:3-9.
7. Landay AL, Muirhead KA. Procedural guidelines for performing immunophenotyping by flow cytometry. *Clin Immunol Immunopath.* 1989;52:48-60.
8. Centers for Disease Control. Update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *MMWR.* 1988;37:377-388.
9. *Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue: Tentative Guideline.* Villanova, PA: National Committee for Clinical Laboratory Standards; 1991. NCCLS document M29-T2.
10. *Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes; Tentative Guideline.* Villanova, PA: National Committee for Clinical Laboratory Standards; 1992. NCCLS document H42-T.
11. *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture: Approved Standard.* Villanova, PA: National Committee for Clinical Laboratory Standards; 1991. NCCLS document H3-A3.
12. Prince HE, Hirji K, Waldbeser LS, Plaeger-Marshall S, Kleinman S, Lanier LL. Influence of racial background on the distribution of T-cell subsets and Leu 11-positive lymphocytes in healthy blood donors. *Diagn Immunol.* 1985;3(1):33-37.
13. *How to Define and Determine Reference Intervals in the Clinical Laboratory: Approved Guideline.* Villanova, PA: National Committee for Clinical Laboratory Standards; 1992. NCCLS document C28-A.
14. Nicholson JKA, Rao PE, Calvelli T, et al. Artifactual staining of monoclonal antibodies in two-color combinations is due to an immunoglobulin in the serum and plasma. *Cytometry.* 1994;18:140-146.

WARRANTY

The product sold hereunder is warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, that extend beyond the description on the label of the product. BD's sole liability is limited to either replacement of the products or refund of the purchase price. BD is not liable for property damage, personal injury, or economic loss caused by the product.

Becton, Dickinson and Company
BD Biosciences
San Jose, CA 95131
Tel 877.232.8995
Fax 408.954.2347
ClinicalApplications@bd.com

EC REP **BENEX Limited**
Rineanna House
Shannon Free Zone
Shannon, County Clare
Ireland
Tel 353.61.472920
Fax 353.61.472907

BD Biosciences
European Customer Support
Tel 32.2.400.9895
Fax 32.2.401.7094
help.biosciences@europe.bd.com

bdbiosciences.com