BD FACS™ Lysing Solution

100 mL— Catalog No. 349202

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Symbols glossary website:
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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.

BD FACS Lysing Solution is appropriate for use with reagents such as BD Tritest™ or BD 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™[1] 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 BD FACS Lysing Solution, which lyses erythrocytes under

1. Ficoll-Paque is a trademark of GE Healthcare.

gentle hypotonic conditions while preserving the leucocytes.

4. REAGENT

Reagent Provided

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

Precautions

- For In Vitro Diagnostic Use.

BD FACS Lysing Solution contains 25 – <50% 2,2′-oxybisethanol (diethylene glycol), CAS number 111-46-6; 5 – <10% formaldehyde, CAS number 50-00-0; and 3 – <5% methanol, CAS number 67-56-1. The lysing solution is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Visit regdocs.bd.com to download the Safety Data Sheet.

	Danger
	H302+H312+H332: Harmful if swallowed, in contact with skin or if inhaled. H315: Causes skin irritation. H319: Causes serious eye irritation. H317: May cause an allergic skin reaction. H341: Suspected of causing genetic defects. H350: May cause cancer. H371: May cause damage to organs. H335: May cause respiratory irritation. H373: May cause damage to organs through prolonged or repeated exposure. P201: Obtain special instructions before use. P202: Do not handle until all safety precautions have been read and understood. P260: Do not breathe dust/fume/gas/mist/vapors/spray. P264: Wash thoroughly after handling. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P337+P313: If eye irritation persists: Get medical advice/attention. P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P312: Call a POISON CENTER/doctor if you feel unwell. P308+P313: If exposed or concerned: Get medical advice/attention

For the USA, the following warnings apply in addition to the ones shown previously:

	Danger
 	H402: Harmful to aquatic life. P271: Use only outdoors or in a well-ventilated area. P273: Avoid release to the environment. P270: Do not eat, drink or smoke when using this product. P281: Use personal protective equipment as required. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302+P352: IF ON SKIN: Wash with plenty of water. P301+P312: IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. P330: Rinse mouth. P321: Specific treatment (see on this label). P363: Wash contaminated clothing before reuse. P405: Store locked up. P403: Store in a well-ventilated place. P233: Keep container tightly closed. P501: Dispose of contents/container to an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[8,9] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear and gloves.

Dilution Instructions

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

5. STORAGE AND HANDLING

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

6. INSTRUMENT

BD 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). We recommend a BD FACSCalibur™, BD FACSCanto™, or BD FACSCanto™ II flow cytometer. However, results may be achieved using other platforms. Refer to the appropriate reagent instructions for use (IFU) for specific instrument limitations.

7. SPECIMEN COLLECTION AND PREPARATION

Collect blood aseptically by venipuncture[10,11] into a sterile BD Vacutainer® EDTA 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°C–25°C) until ready for staining and lysing. Refer to the appropriate reagent IFU for storage restrictions prior to staining. See Section 10, Limitations, for possible interfering conditions.

8. PROCEDURE

Reagent Provided

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

Reagents and Materials Required but Not Provided

- 1X BD FACS Lysing Solution, diluted as indicated in Section 4, Reagent: Dilution Instructions
- BD Vacutainer EDTA blood collection tubes, or equivalent
- Disposable 12 x 75-mm capped polystyrene test tubes
- BD monoclonal antibodies to human leucocyte antigens (for example, BD Tritest or BD Simultest reagents)
- Vortex mixer
- Micropipettor with tips

Other materials can be required. Refer to the appropriate reagent IFU for more information.

Preparing Samples

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

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

9. RESULTS

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

Figure 1 CD45 vs SSC and CD3 vs CD4 dot plots obtained with BD Tritest reagent

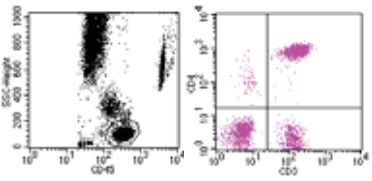
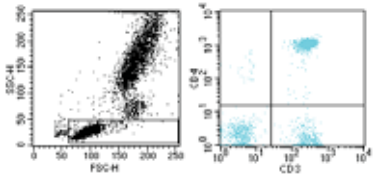


Figure 2 FSC vs SSC and CD3 vs CD4 dot plot obtained with BD Simultest reagent



10. LIMITATIONS

- Laboratories must establish their own normal reference ranges for each reagent parameter that can be affected by sex of patient, age of patient, and preparative technique. Race of patient may also have an effect,[12] although sufficient data is 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.
- BD FACS Lysing Solution is specifically formulated for use with BD FACS™ brand flow cytometers.
- EDTA is the anticoagulant of choice. BD has limited information concerning use of other anticoagulants such as heparin.
- Retain samples in BD Vacutainer EDTA blood collection tubes at room temperature (20°C–25°C) prior to staining and lysing. Refer to the reagent IFU for maximum storage times after collection.
- Samples with nucleated red blood cells may show incomplete lysis of red blood cells because BD 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]
- When using monoclonal reagents that react with serum immunoglobulins, blood samples should be washed with 1X phosphate buffered saline (PBS) or physiological saline prior to staining and lysing.[14]
- A monoclonal reagent against a cell surface antigen or receptor that is: a) shed into plasma (for example, IL-2 receptor) or b) occupied by plasma components (for example, complement receptors) can have reduced staining intensity when analyzed with the lysed whole blood methodology.

11. 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 following table. 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	18–70	84	43	29–57
	Female	18–70	75	46	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.

12. PERFORMANCE CHARACTERISTICS

Precision

For the BD 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 BD FACScan flow cytometers.

Subjects	Lymphocyte Subset	n	Mean	SD[a]	df[b]
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. SD = standard deviation
b. df = degrees of freedom: the number of observations (3) minus the number of means (1) multiplied by the number of subjects (n).

For the BD 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 BD Simultest™ IMK-Lymphocyte reagent panel and each aliquot was run twice on the same BD FACScan flow cytometer.

Subjects	Lymphocyte Subset	n	Mean[a]	SD[b]	df[c]
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 (the mean of the individual means).
b. SD = standard deviation
c. 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 BD FACS Lysing Solution, washed, and analyzed for white cell recovery using the Ortho 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 BD FACS Lysing Solution, washed, and analyzed for residual red blood cells using the Ortho ELT-1500 Clinical Hematology Analyzer. No red blood cells were detected.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. 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 OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

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