BD Pharmingen™

Technical Data Sheet

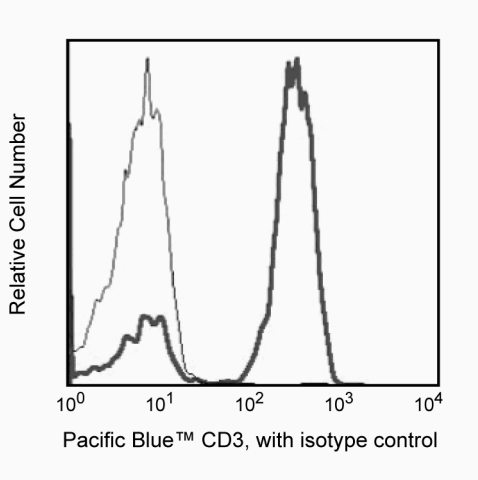
Pacific Blue™ Mouse Anti-Human CD3

Product Information

Material Number:	**558117**
Alternate Name:	CD3e; CD3E; T3E; TCRE; T-cell surface antigen T3/Leu-4 epsilon
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	UCHT1
Immunogen:	Human infant thymocytes and peripheral blood lymphocytes from a Sézary Syndrome donor
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	III 471
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

Description

The UCHT1 monoclonal antibody specifically binds to the human CD3ε-chain, a 20-kDa subunit of the CD3/T cell antigen receptor complex. CD3ε is expressed on 70-80% of normal human peripheral blood lymphocytes and 60-85% of thymocytes. Studies from the HLDA Workshop show that this antibody is mitogenic for CD3ε-positive cells when used in conjunction with costimulatory agents such as pokeweed mitogen or anti-CD28 antibody. CD3 plays a central role in signal transduction during antigen recognition. The UCHT1 antibody stains both surface and intracellular CD3ε unlike the other CD3 clone, HIT3a, that stains only extracellular CD3ε.



Flow cytometric analysis of CD3 expression on human peripheral blood lymphocytes. *Whole blood was stained with either Pacific Blue™ Mouse IgG1, κ Isotype Control (Cat. No. 558120; dashed line histogram) or Pacific Blue™ Mouse Anti-Human CD3 (Cat. No. 558117; solid line histogram). Erythrocytes were lysed with Lysing Buffer (Cat. No. 555899). Fluorescence histograms were derived from events with the forward and side light-scattering characteristics of viable lymphocytes.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody is conjugated to Pacific Blue™ under optimum conditions, and unreacted Pacific Blue™ was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558120	Pacific Blue™ Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. Pacific Blue™ has a maximum absorption of 416 nm and maximum emission of 451 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
7. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Barclay NA, Brown MH, Birkeland ML, et al, ed. *The Leukocyte Antigen FactsBook.* San Diego, CA: Academic Press; 1997(Biology)

Beverley PC, Callard RE. Distinctive functional characteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody. *Eur J Immunol.* 1981; 11(4):329-334. (Biology)

Knapp W. W. Knapp .. et al., ed. *Leucocyte typing IV : white cell differentiation antigens.* Oxford New York: Oxford University Press; 1989:1-1182. (Clone-specific)

Lanier LL, Allison JP, Phillips JH. Correlation of cell surface antigen expression on human thymocytes by multi-color flow cytometric analysis: implications for differentiation. *J Immunol.* 1986; 137(8):2501-2507. (Biology)

McMichael AJ. A.J. McMichael .. et al., ed. *Leucocyte typing III : white cell differentiation antigens.* Oxford New York: Oxford University Press; 1987:1-1050. (Clone-specific)

Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Biology)

Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Clone-specific)

Monoclonal Antibodies Detecting Human Antigens

CD10 (HI10a)

Form	Catalog number
FITC	340925
PE	340921
PE-Cy7	341092
APC	340923
APC-H7	655404
APC-R700	659120

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

RESEARCH APPLICATIONS

Research applications include:

- Characterization of non-T (common) acute lymphoblastic leukemias[1,2]
- Analysis of early stages of hematopoietic differentiation[3–5]
- Analysis of neutrophil chemotaxis[6–8]

DESCRIPTION

Specificity

The CD10 antibody recognizes a 100-kilodalton (kDa) type II transmembrane, glycosylated, zinc-containing metalloprotease.[9,10] The CD10 antigen is also known as common acute lymphoblastic leukemia antigen (CALLA), neutral endopeptidase (NEP), gp100, and enkephalinase.[11]

Antigen distribution

The CD10 antigen is found on lymphocytes from samples with acute B-lymphoid leukemia.[12] The CD10 antigen is also present on a wide variety of normal and neoplastic cell types including renal epithelium, fibroblasts, granulocytes, germinal center B lymphocytes,[13] neutrophils,[6,7,14] some T-cell leukemias,[15] and some lymphoma, melanoma, and glioma cell lines.[11]

The CD10 antigen cleaves a number of biologically active peptides,[16] including fMLP, and may modulate the chemotactic activity of fMLP towards neutrophils.[8] Inhibition of the CD10 antigen promotes B-cell maturation,[17] suggesting that it plays a role in B-cell development.

Clone

The CD10 antibody, clone HI10a,[10] is derived from the hybridization of P3-63-Ag8.653 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with blasts from a patient with acute CALLA leukemia.

Composition

The CD10 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following are supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	50	20	12.5	1	12.5	Gelatin	0.1% Sodium azide
PE	50	20	6	1	6	Gelatin	0.1% Sodium azide
PE-Cy™7	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide
APC	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

bdbiosciences.com
ResearchApplications@bd.com

6/2015 23-4824-04

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
APC-H7	100	5	25	0.5	50	BSA	ProClin® 300
APC-R700[b]	100	5	6.25	0.5	12.5	BSA	ProClin 300

a. Volume required to stain 10^6 cells.
b. BD Horizon™ APC-R700

CAUTION Some PE-Cy7, APC-H7, and APC-R700 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

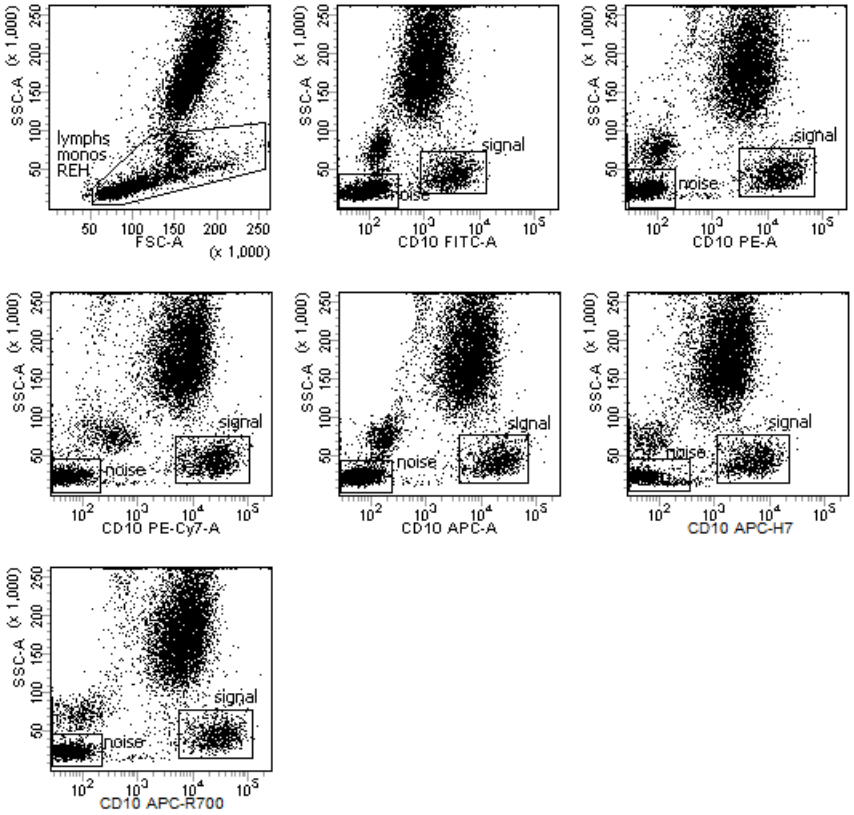
PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on 10^6 REH cells added per mL of whole blood stained with the indicated conjugated antibody and gated on lymphocytes, monocytes, and REH cells. Laser excitation was at 488 nm, 635 nm, or 640 nm.

The APC-R700 conjugate is read off the red laser (640 nm) using a 685 longpass mirror with a 712/21 bandpass filter. Representative data analyzed with a BD FACS™ brand flow cytometer is shown in the following plots.



HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[18,19] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing and gloves.

Some reagents are bottled with ProClin 300, and contain 0.003% of a mixture of CMIT/MIT (3:1), CAS number 55965-84-9.

Warning

H317 May cause an allergic skin reaction.

Wear protective gloves/eye protection. Wear protective clothing. Avoid breathing mist/vapours/spray. If skin irritation or rash occurs: Get medical advice/attention. IF ON SKIN: Wash with plenty of water. Dispose of contents/container in accordance with local/regional/national/international regulations.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

REFERENCES

1. Hann IM, Richards SM, Eden OB, Hill FGH. Analysis of the immunophenotype of children treated on the Medical Research Council United Kingdom Acute Lymphoblastic Leukaemia Trial XI (MRC UKALLXI). *Leukemia*. 1998;12:1249-1255.
2. Nadler LM, Korsmeyer SJ, Anderson KC, et al. B cell origin of non-T cell acute lymphoblastic leukemia: a model for discrete stages of neoplastic and normal pre-B cell differentiation. *J Clin Invest*. 1984;74:332-340.
3. Dworzak MN, Fritsch G, Fleischer C, et al. Multiparameter phenotype mapping of normal and post-chemotherapy B lymphopoiesis in pediatric bone marrow. *Leukemia*. 1997;11:1266-1273.
4. Dworzak MN, Fritsch G, Fröschl G, Printz D, Gadner H. Four-color flow cytometric investigation of terminal deoxynucleotidyl transferase–positive lymphoid precursors in pediatric bone marrow: CD79a expression precedes CD19 in early B-cell ontogeny. *Blood*. 1998;92:3203-3209.
5. Loken MR, Shah VO, Dattilio KL, Civin CI. Flow cytometric analysis of human bone marrow. II. Normal B-lymphocyte development. *Blood*. 1987;70:1316-1324.
6. Connelly JC, Skidgel RA, Schulz WW, Johnson AR, Erdos EG. Neutral endopeptidase 24.11 in human neutrophils: cleavage of chemotactic peptide. *Proc Natl Acad Sci USA*. 1985;82:8737-8741.
7. Erdös EG, Wagner B, Harbury CB, Painter RG, Skidgel RA, Fa XG. Down-regulation and inactivation of neutral endopeptidase 24.11 (enkephalinase) in human neutrophils. *J Biol Chem*. 1989;264:14519-14523.
8. Hofman P, Selva E, Le Negrate G, et al. CD10 inhibitors increase f-Met-Leu-Phe-induced neutrophil transmigration. *J Leukoc Biol*. 1998;63:312-320.
9. Greaves MF. Monoclonal antibodies as probes for leukemic heterogeneity and hematopoietic differentiation. In: Knapp W, ed. *Leukemia Markers*. New York, NY: Academic Press; 1981:19.
10. Zola H. CD10 Workshop Panel report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens*. Vol 1. New York, NY: Oxford University Press; 1995:505-507.
11. Letarte M, Vera S, Tran R, et al. Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. *J Exp Med*. 1988;168:1247-1253.

12. LeBien TW, McCormack RT. The common acute lymphoblastic leukemia antigen (CD10)—emancipation from a functional enigma. *Blood*. 1989;73:625-635.

13. Caligaris-Cappio F, Riva M, Tesio L, Schena M, Gaidano G, Bergui L. Human normal $CD5^+$ B lymphocytes can be induced to differentiate to $CD5^-$ B lymphocytes with germinal center cell features. *Blood*. 1989;73:1259-1263.

14. Connelly JC, Chambless R, Holiday D, Chittenden K, Johnson AR. Up-regulation of neutral endopeptidase (CALLA) in human neutrophils by granulocyte-macrophage colony-stimulating factor. *J Leukoc Biol*. 1993;53:685-690.

15. Consolini R, Legitimo A, Rondelli R, et al. Clinical relevance of CD10 expression in childhood ALL. *Haematologica*. 1998;83:967-973.

16. Erdös EG, Skidgel RA. Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. *FASEB J*. 1989;3:145-151.

17. Salles G, Rodewald HR, Chin BS, Reinherz EL, Shipp MA. Inhibition of CD10/neutral endopeptidase 24.11 promotes B-cell reconstitution and maturation in vivo. *Proc Natl Acad Sci USA*. 1993;90:7618-7622.

18. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29-A3.

19. Centers for Disease Control. Perspectives in disease prevention and health promotion 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.

PATENTS AND TRADEMARKS

Technical Data Sheet

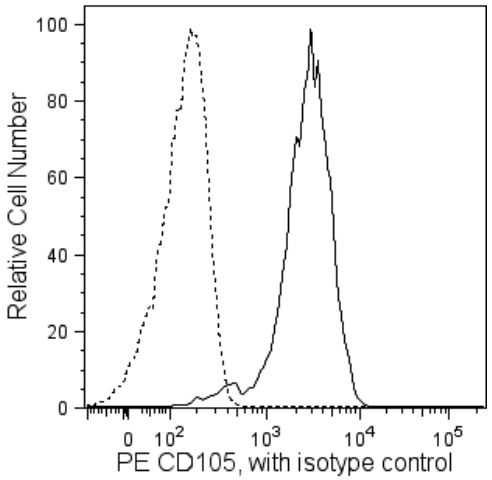
PE Mouse anti-Human CD105

Product Information

Material Number:	**560839**
Alternate Name:	END; Endoglin; HHT1; MSC; ORW; ORW1
Size:	100 Tests
Vol. per Test:	5 µl
Clone:	266
Immunogen:	Human Umbilical Vein Endothelial Cells
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The 266 monoclonal antibody specifically binds to CD105. CD105 is a type I transmembrane glycoprotein that is encoded by *END* (Endoglin) and belongs to the transforming growth factor-β (TGF-β) type III receptor family. CD105 is expressed on cells as a homodimer comprised of ~95 kDa subunits. CD105 is expressed on vascular endothelial cells and placental syncytiotrophoblasts and at lower levels on stromal fibroblasts. It is also expressed on mesenchymal stem cells, erythroid precursors, activated macrophages, pre-B cells, and some tumor cells and cell lines including U937 cells. CD105 serves as a regulatory component of the TGF-β receptor system. In association with TGF-βRI or TGF- βRII, CD105 binds TGF-β1 and TGF-β3 with high affinity but does not bind to TGF-β2. Expression of CD105 is increased on activated endothelium in tissues undergoing angiogenesis, such as in tumors, or in cases of wound healing or dermal inflammation.



Flow cytometric analysis of CD105 expression on human U937 cells. *U937 cells (ATCC, Cat No. CRL-1593.2) were stained with PE Mouse anti-Human CD105 antibody (Cat. No. 5060839; solid line histogram) or a PE mIgG1, κ isotype control (Cat. No. 554680; dashed line histogram). Flow cytometric fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of viable cells. Flow cytometry was performed using a BD LSR™ II flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Flow cytometry	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
7. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

References

Gougos A, Letarte M. Identification of a human endothelial cell antigen with monoclonal antibody 44G4 produced against a pre-B leukemic cell line. *J Immunol.* 1988; 141(6):1925-1933. (Biology)

Lastres P, Bellon T, Cabañas C, et al. Regulated expression on human macrophages of endoglin, an Arg-Gly-Asp-containing surface antigen. *Eur J Immunol.* 1992; 22(2):393-397. (Biology)

Tomchuck SL, Zwezdaryk KJ, Coffelt SB, Waterman RS, Danka ES, Scandurro AB. Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses. *Stem Cells.* 2008; 26(1):99-109. (Clone-specific: Flow cytometry)

Wang JM, Kumar S, Pye D, van Agthoven AJ, Krupinski J, Hunter RD. A monoclonal antibody detects heterogeneity in vascular endothelium of tumours and normal tissues. *Int J Cancer.* 1993; 54(3):363-370. (Biology)

Westphal JR, Willems HW, Schalkwijk CJ, Ruiter DJ, de Waal RM. A new 180-kDa dermal endothelial cell activation antigen: in vitro and in situ characteristics. *J Invest Dermatol.* 1993; 100(1):27-34. (Biology)

Monoclonal Antibodies Detecting Human Antigens

CD14 (MϕP9)

Form	Catalog number	Form	Catalog number
Pure	347490	APC	340436
FITC	347493	APC-Cy7	333945
PE	347497	APC-H7	641394
PerCP	340585	V500-C	647459

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

RESEARCH APPLICATIONS

Research applications include:

- Identification and enumeration of mature monocytes in peripheral blood
- Study of monocyte-derived dendritic cells[1-3]
- Study of inflammatory disease[4]

DESCRIPTION

Specificity

The CD14 antibody recognizes a human monocyte/macrophage antigen with a molecular weight of 55 kilodaltons (kDa).[5]

Antigen distribution

The CD14 antigen is present on the majority of normal peripheral blood monocytes.[6] CD14 has weak reactivity with peripheral blood granulocytes.[7]

Clone

The CD14 antibody, clone MϕP9,[8,9] is derived from hybridization of Sp2/0 mouse myeloma cells with spleen cells from BALB/c mice immunized with peripheral blood monocytes from a patient with rheumatoid arthritis.

Composition

The CD14 antibody is composed of mouse IgG_{2b} heavy chains and kappa light chains.

Product configuration

The following are supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
Pure	100	20	50.0	2.0	25	Gelatin	0.1% Sodium azide
FITC	100	20	50.0	2.0	25	Gelatin	0.1% Sodium azide
PE	100	20	100.0	2.0	50	Gelatin	0.1% Sodium azide
PerCP	50	20	25.0	1.0	25	Gelatin	0.1% Sodium azide
APC	100	5	25.0	0.5	50	Gelatin	0.1% Sodium azide
APC-Cy™7	100	5	25.0	0.5	50	Gelatin	0.1% Sodium azide
APC-H7	100	5	12.5	0.5	25	BSA	ProClin™ 300
V500-C[b]	100	5	50.0	0.5	100	BSA	ProClin™ 950

a. Volume required to stain 10^6 cells.
b. BD Horizon™ V500-C.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

bdbiosciences.com
ResearchApplications@bd.com

06/2018 23-1323-16

CAUTION Some APC-Cy7 conjugates, and to a lesser extent APC-H7 conjugates, show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

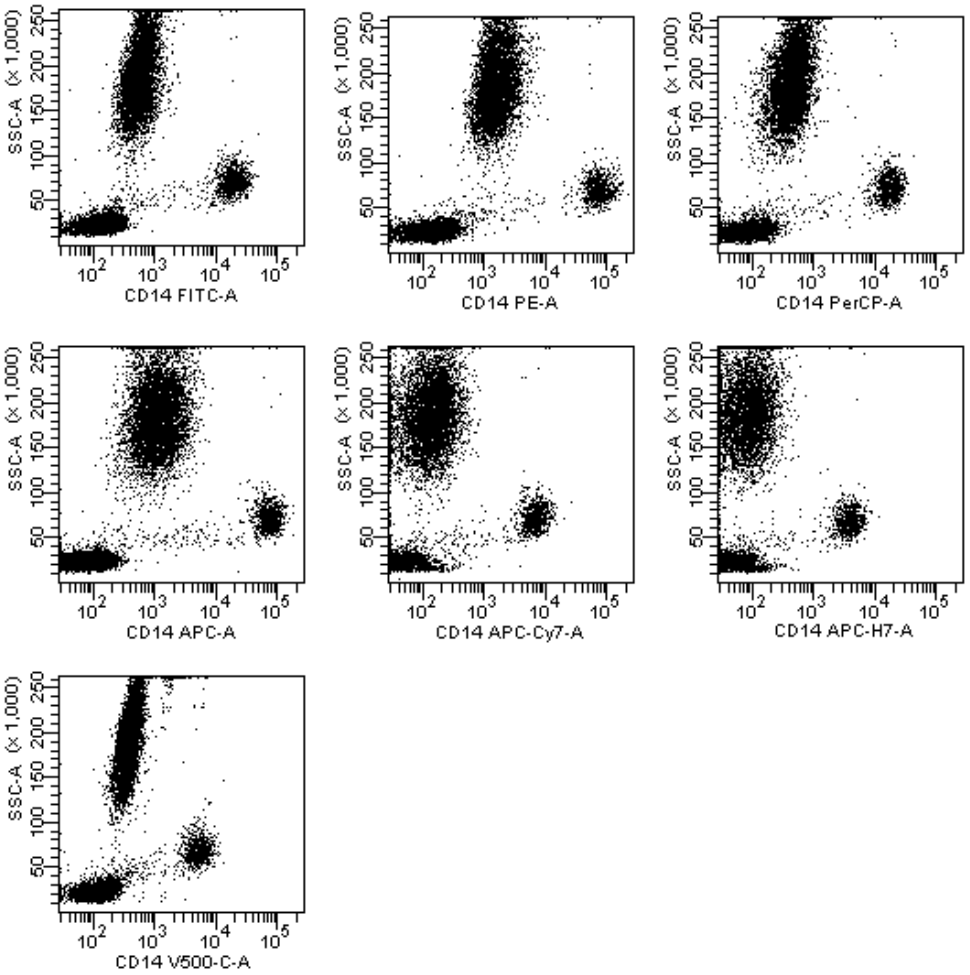
CAUTION Prolonged exposure of cells to paraformaldehyde can lead to increased autofluorescence in the violet channels. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 405 nm, 488 nm, or 635 nm. Representative data analyzed with a BD FACS™ brand flow cytometer is shown in the following plots.



HANDLING AND STORAGE

Store vials at 2°C – 8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[10,11] 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.

Some reagents are bottled with ProClin 300, and contain 0.003% of a mixture of CMIT/MIT (3:1), CAS number 55965-84-9.

Warning

H317 May cause an allergic skin reaction.

Wear protective gloves/eye protection. Wear protective clothing. Avoid breathing mist/vapours/spray. If skin irritation or rash occurs: Get medical advice/attention. IF ON SKIN: Wash with plenty of water. Dispose of contents/container in accordance with local/regional/national/international regulations.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

REFERENCES

1. Li G, Kim YJ, Mantel C, Broxmeyer HE. P-selectin enhances generation of $CD14^{+}CD16^{+}$ dentritic-like cells and inhibits macrophage maturation from human peripheral blood monocytes. *J Immunol.* 2003;171:669–677.
2. Chomarat P, Dantin C, Bennett L, Banchereau J, Palucka AK. TNF skews monocyte differentiation from macrophages to dendritic cells. *J Immunol.* 2003;171:2262–2269.
3. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. *Annu Rev Immunol.* 2000;18:767–811.
4. Scherberich JE, Nockher WA. $CD14^{++}$ monocytes, $CD14^{+}/CD16^{+}$ subset and soluble CD14 as biological markers of inflammatory systemic diseases and monitoring immunosuppressive therapy. *Clin Chem Lab Med.* 1999;37:209–213.
5. Goyert SM, Ferrero E. Biochemical analysis of myeloid antigens and cDNA expression of gp55 (CD14). In: McMichael AJ, ed. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:613–619.
6. Bernstein ID, Self S. Joint report of the myeloid section of the Second International Workshop on Human Leukocyte Differentiation Antigens. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human Myeloid and Hematopoietic Cells.* Vol 3. New York, NY: Springer-Verlag; 1986:1–25.
7. Jayaram Y, Hogg N. Surface expression of CD14 molecules on human neutrophils. In: Knapp W, Dörken B, Gilks WR, et al., eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1989:796–797.
8. Dimitriu-Bona A, Burmester GR, Waters SJ, Winchester RJ. Human mononuclear phagocyte differentiation antigens. I. Patterns of antigenic expression on the surface of human monocytes and macrophages defined by monoclonal antibodies. *J Immunol.* 1983;130:145–152.
9. Herrmann F, Komischke B, Odenwald E, Ludwig WD. Use of monoclonal antibodies as a diagnostic tool in human leukemia. I. Acute myeloid leukemia and acute phase of chronic myeloid leukemia. *Blut.* 1983;47:157–163.

10. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29-A3.

11. Centers for Disease Control. Perspectives in disease prevention and health promotion 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.

PATENTS AND TRADEMARKS

BD CD16 (CLB/FcGran1)

Monoclonal Antibodies Detecting Human Antigens

Form	Catalog number
FITC	656146

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include:

- Enumeration of natural killer (NK) cells[1]
- Delineation of NK cell activation and signal transduction[2]
- Investigation of antibody-dependent cellular cytotoxicity (ADCC)[3]
- Characterization of leukemias and lymphomas[4]

Description

Specificity

The CD16 antibody recognizes a 50–65 kilodalton (kDa) transmembrane glycoprotein which is a member of the Fcγ receptor subfamily of the immunoglobulin (Ig) superfamily. The antigen is also known as FcγRIIIa.[5]

Antigen distribution

The CD16 antigen is expressed on approximately 15% of peripheral blood lymphocytes and is present on virtually all resting NK lymphocytes.[6] The CD16 antigen can be expressed on $CD3^+$ T lymphocytes from certain individuals.[7] The CD16 antigen is also expressed on macrophages, granulocytes, and neutrophils.[5,8] A variable number of $CD16^+$ lymphocytes co-express either the CD57 antigen or low-density CD8 antigen, or both.[8-10] $CD16^+CD56^+$ NK cells and dendritic cells demonstrate a reciprocal activation of one another.[11]

Clone

The CD16 antibody, clone CLB/FcGran1,[12] is derived from the hybridization of Sp2/0 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with human granulocytes.

Composition

The CD16 antibody is composed of mouse IgG_{2a} heavy chains and kappa light chains.

Product configuration

The following is supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	50	20	50	1	50	Gelatin	0.1% Sodium azide

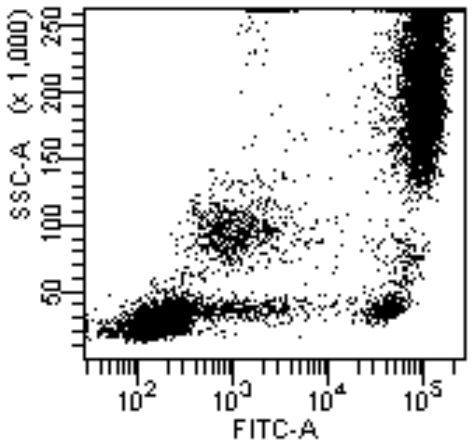
Procedure

Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Representative Data

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 488 nm. Representative data analyzed with a BD flow cytometer is shown in the following plot.



Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Warning

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[13,14] 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.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

References

1. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol.* 2001;22:633-640.

2. Galandrini R, Tassi I, Mattia G, et al. SH2-containing inositol phosphatase (SHIP-1) transiently translocates to raft domains and modulates CD16-mediated cytotoxicity in human NK cells. *Blood.* 2002;100:4581-4589.

3. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. *Nat Rev Immunol.* 2008;8:34-47.

4. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.

5. Zola H, Swart B, Nicholson I, Voss E. *Leukocyte and Stromal Cell Molecules: The CD Markers.* Hoboken, NJ: John Wiley & Sons, Inc.; 2007.

6. Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J Immunol.* 1986;136:4480-4486.

7. Lanier LL, Kipps T, Phillips JH. Functional properties of a unique subset of cytotoxic $CD3^+$ T lymphocytes that express Fc receptors for IgG (CD16/Leu-11 antigen). *J Exp Med.* 1985;162:2089-2106.

8. Lanier LL, Le AM, Phillips JH, Warner NL, Babcock GF. Subpopulations of human natural killer cells defined by expression of the Leu-7 (HNK-1) and Leu-11 (NK-15) antigens. *J Immunol.* 1983;131:1789-1796.

9. Perussia B, Starr S, Abraham S, Fanning V, Trinchieri G. Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. I. Characterization of the lymphocyte subset reactive with B73.1. *J Immunol.* 1983;130:2133-2141.

10. Perussia B, Trinchieri G, Jackson A, et al. The Fc receptor for IgG on human natural killer cells: phenotypic, functional, and comparative studies with monoclonal antibodies. *J Immunol.* 1984;133:180-189.

11. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between natural killer cells and dendritic cells. *J Exp Med.* 2002;195:327-333.

12. Schmidt RE, Perussia B. Cluster report: CD16. In: Knapp W, Dörken B, Gilks WR, et al., eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:574-578.

13. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.

14. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

Patents and Trademarks

For US patents that may apply, see bd.com/patents.

Contact Information

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

bdbiosciences.com
ResearchApplications@bd.com

Monoclonal Antibodies Detecting Human Antigens

CD19 (SJ25C1)

Form	Catalog number	Form	Catalog number
FITC	340409	APC-R700	659121
PE	340364	APC-Cy7	348794
PerCP	340421	APC-H7	641395
PerCP-Cy5.5	340951	AmCyan	339190
PE-Cy7	341093	V450	644491
APC	340437		

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

RESEARCH APPLICATIONS

Research applications include:

- Studies of B-lymphocyte proliferation and activation[1,2]
- Enumeration of B-lymphocytes in peripheral blood[3]
- Research on B-lymphocyte neoplasms[4]
- Investigation into B-cell differentiation in bone marrow[5]
- Examination of B-lymphocyte apoptosis[6]

DESCRIPTION

Specificity

The CD19 antibody recognizes a 90-kilodalton (kDa) antigen that is present on human B lymphocytes.[7,8]

Antigen distribution

The CD19 antigen is present on approximately 7% to 23% of human peripheral blood lymphocytes[9] and on splenocytes.[10] CD19 is reactive with the B-lymphocyte areas of normal tonsil and lymph nodes.[3] The CD19 antigen is present on human B lymphocytes at all stages of maturation.[3] CD19 does not react with resting or activated T lymphocytes, granulocytes, or monocytes.[5]

Clone

The CD19 antibody, clone SJ25C1,[8] is derived from the hybridization of Sp2/0 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with NALM1 and NALM16 cells.

Composition

The CD19 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following are supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	50	20	6	1	6	Gelatin	0.1% Sodium azide
PE	50	20	12.5	1	12.5	Gelatin	0.1% Sodium azide
PerCP	50	20	12.5	1	12.5	Gelatin	0.1% Sodium azide
PerCP-Cy™5.5	50	20	5	1	5	Gelatin	0.1% Sodium azide
PE-Cy™7	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide
APC	100	5	25	0.5	50	Gelatin	0.1% Sodium azide
APC-R700[b]	100	5	6.25	0.5	12.5	BSA	ProClin™ 300

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

bdbiosciences.com
ResearchApplications@bd.com

03/2019 23-2944-13

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
APC-Cy7	100	5	25	0.5	50	Gelatin	0.1% Sodium azide
APC-H7	100	5	25	0.5	50	Gelatin	0.1% Sodium azide
AmCyan	100	5	12.5	0.5	25	BSA	0.1% Sodium azide
V450[b]	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide

a. Volume required to stain 10^6 cells.
b. BD Horizon™ APC-R700, BD Horizon™ V450.

CAUTION Some APC-Cy7 conjugates, and to a lesser extent PE-Cy7, APC-H7, and APC-R700 conjugates, show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

CAUTION Prolonged exposure of cells to paraformaldehyde can lead to increased autofluorescence in the violet channels. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

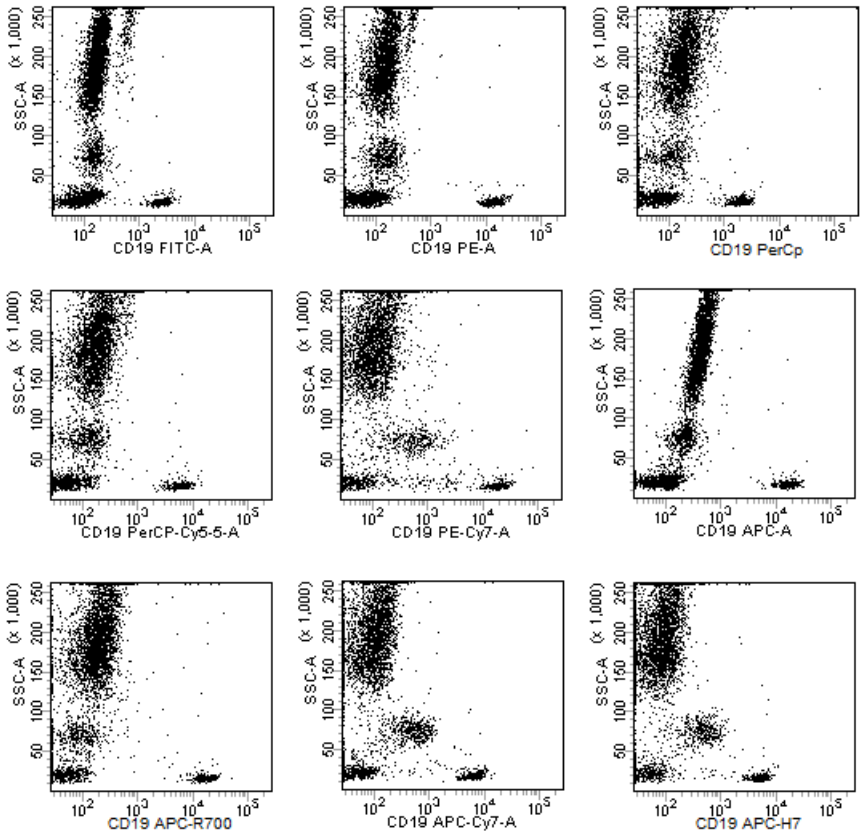
PROCEDURE

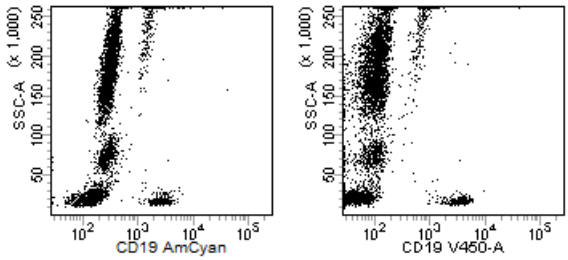
Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 405 nm, 488 nm, 635 nm, or 640 nm.

The APC-R700 conjugate is read off the red laser (640 nm) using a 685 longpass mirror with a 712/21 bandpass filter. Representative data analyzed with a BD FACS™ brand flow cytometer is shown in the following plots.





HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[11,12] 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.

Some reagents are bottled with ProClin 300, and contain 0.003% of a mixture of CMIT/MIT (3:1), CAS number 55965-84-9.

Warning

H317 May cause an allergic skin reaction.

Wear protective gloves/eye protection. Wear protective clothing. Avoid breathing mist/vapours/spray. If skin irritation or rash occurs: Get medical advice/attention. IF ON SKIN: Wash with plenty of water. Dispose of contents/container in accordance with local/regional/national/international regulations.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

REFERENCES

1. Lewis DE, Cook RG, Ditmore RD, et al. Effects of monoclonal antibodies against CD19, CD20, and a new B-cell antigen, 37.1, on B-cell proliferation and differentiation. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:452-453.
2. Pezzutto A, Dörken B, Rabinovitch PS, Ledbetter JA, Moldenhauer G, Clark EA. CD19 monoclonal antibodies inhibit B-cell activation and proliferation. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:358-360.
3. Dörken B, Möller P, Pezzutto A, Schwartz-Albiez R, Moldenhauer G. B-cell antigens: CD19. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1989:34-36.
4. Ling NR, Maclennan ICM, Mason DY. B-cell and plasma cell antigens: new and previously defined clusters. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:302-335.
5. Loken MR, Shah VO, Dattilio KL, Civin CI. Flow cytometric analysis of human bone marrow. II. Normal B-lymphocyte development. *Blood*. 1987;70:1316-1324.

6. Mongini PK, Jackson AE, Tolani S, Fattah RJ, Inman JK. Role of complement-binding CD21/CD19/CD81 in enhancing human B cell protection from Fas-mediated apoptosis. *J Immunol.* 2003;171:5244-5254.

7. Moldenhauer G, Dörken B, Schwartz R, Pezzutto A, Knops J, Hammerling GJ. Analysis of ten B lymphocyte-specific workshop monoclonal antibodies. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human B Lymphocytes*. Vol 2. New York, NY: Springer-Verlag; 1986:61-67.

8. Nadler LM. B Cell/Leukemia Panel Workshop: Summary and Comments. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human B Lymphocytes*. Vol 2. New York, NY: Springer-Verlag; 1986:3-43.

9. Reichert T, DeBruyère M, Deneys V, et al. Lymphocyte subset reference ranges in adult Caucasians. *Clin Immunol Immunopathol.* 1991;60:190-208.

10. Tedder TF, Zhou LJ, Engel P. The CD19/CD21 signal transduction complex of B lymphocytes. *Immunol Today.* 1994;15:437-442.

11. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29-A3.

12. Centers for Disease Control. Perspectives in disease prevention and health promotion 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.

PATENTS AND TRADEMARKS

Monoclonal Antibodies Detecting Human Antigens

CD21 (B-ly4)

Form	Catalog number
V450	658169

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

RESEARCH APPLICATIONS

Research applications include:

- Investigation of lymphocyte transformation by Epstein-Barr virus (EBV)[1]
- Analysis of the complement system[2]
- Examination of B-cell activation and survival[3-5]
- Characterization of leukemias and lymphomas[6]

DESCRIPTION

Specificity

The CD21 antibody specifically binds to a 145-kilodalton (kDa) glycosylated type I integral membrane protein.[7] The CD21 antigen is also known as complement receptor type 2 (CR2), C3d receptor, and Epstein-Barr virus receptor (EBV-R).

Antigen distribution

The CD21 antigen is expressed on mature B cells,[8] marginal zone B cells,[9] follicular dendritic cells,[10] and some epithelial cells.[7] The CD21 antigen is expressed at low levels on some subsets of thymocytes and T cells.[7]

The CD21 antigen is a receptor for the C3d fragment of complement[2] and for EBV.[11] The CD21 antigen forms a complex with CD19 and CD81, which provides a co-stimulatory signal for the B-cell receptor.[3-5]

Clone

The CD21 antibody, clone B-ly4,[8] is derived from the hybridization of mouse myeloma cells with spleen cells isolated from immunized mice.

Composition

The CD21 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following is supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
V450[b]	100	5	6.5	0.5	13	Gelatin	0.1% Sodium azide

a. Volume required to stain 10^6 cells.
b. BD Horizon™ V450.

CAUTION Prolonged exposure of cells to paraformaldehyde can lead to increased autofluorescence in the violet channels. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

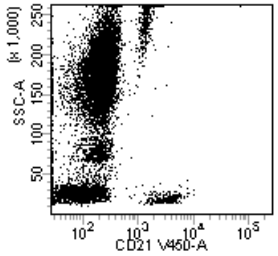
Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

bdbiosciences.com
ResearchApplications@bd.com

6/2015 23-15535-01

REPRESENTATIVE DATA

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 405 nm. Representative data analyzed with a BD FACS™ brand flow cytometer is shown in the following plot.



HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[12,13] 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.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

REFERENCES

1. Boyd AW, Fecondo J. Role of the CD21 antigen in lymphocyte transformation by Epstein-Barr virus. *Immunol Cell Biol.* 1988;66:159-165.
2. Weis JJ, Tedder TF, Fearon DT. Identification of a 145,000 M_r membrane protein as the C3d receptor (CR2) of human B lymphocytes. *Proc Natl Acad Sci USA.* 1984;81:881-885.
3. Cherukuri A, Shoham T, Sohn HW, et al. The tetraspanin CD81 is necessary for partitioning of coligated CD19/CD21-B cell antigen receptor complexes into signaling-active lipid rafts. *J Immunol.* 2004;172:370-380.
4. Fearon DT, Carroll MC. Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. *Annu Rev Immunol.* 2000;18:393-422.
5. Mongini PK, Jackson AE, Tolani S, Fattah RJ, Inman JK. Role of complement-binding CD21/CD19/CD81 in enhancing human B cell protection from Fas-mediated apoptosis. *J Immunol.* 2003;171:5244-5254.
6. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.
7. Zola H, Swart B, Nicholson I, Voss E. *Leukocyte and Stromal Cell Molecules: The CD Markers.* Hoboken, NJ: John Wiley & Sons, Inc.; 2007.
8. Dörken B, Möller P, Pezzutto A, Schwartz-Albiez R, Moldenhauer G. B-cell antigens: CD21. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:58-60.

9. Timens W, Boes A, Poppema S. Human marginal zone B cells are not an activated B cell subset: strong expression of CD21 as a putative mediator for rapid B cell activation. *Eur J Immunol*. 1989;19:2163-2166.

10. Petrasch S, Schmitz J, Perez-Alvarez C, Brittinger G. Reactivity of mAb of the B-cell panel with isolated follicular dendritic cells. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1989:185-187.

11. Fingeroth JD, Weis JJ, Tedder TF, Strominger JL, Biro PA, Fearon DT. Epstein-Barr virus receptor of human B lymphocytes is the C3d receptor CR2. *Proc Natl Acad Sci USA*. 1984;81:4510-4514.

12. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Third Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29-A3.

13. Centers for Disease Control. Perspectives in disease prevention and health promotion 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.

PATENTS AND TRADEMARKS

Monoclonal Antibodies Detecting Human Antigens

CD23 (EBVCS-5)

Form	Catalog number
FITC	656148
PE	341007
APC	340935
APC-R700	659125

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

RESEARCH APPLICATIONS

Research applications include:

- Investigation into the regulation of IgE synthesis[1]
- Examination of B-lymphocyte differentiation[2]
- Analysis of aminoacyl-tRNA-synthetase–interacting multifunctional protein 1 (AIMP1)-induced inflammation[3]
- Characterization of leukemias and lymphomas[4]

DESCRIPTION

Specificity

The CD23 antibody recognizes a 45-kilodalton (kDa) type II membrane glycoprotein, which is a human B-lymphocyte differentiation antigen. The CD23 antigen is also known as the low affinity IgE receptor, Fc epsilon RII, and FcεRII.[2,5–7]

Antigen distribution

The CD23 antigen is present at low density on most normal B lymphocytes[8] and at higher levels on activated B lymphocytes, Epstein-Barr virus (EBV)–transformed lymphoblasts, chronic lymphocytic leukemia (CLL) cells of B-lymphocyte origin, and tonsillar B lymphocytes.[6] The human B-lymphoblastoid cell line, RPMI-8866, releases a 25-kDa species into the culture supernatant.[9]

The CD23 antigen density increases on the surface of B lymphocytes shortly after activation.[10] Expression is induced by interleukin-4 (IL-4) and down-regulated by B-cell growth factor (BCGF).[5,9] The antigen is lost after isotype switching to IgA, IgG, or IgE.[2,9] The CD23 antigen is not present on immature bone marrow B lymphocytes or on T lymphocytes,[2] but it has been reported on monocytes, hypodense eosinophils, and a subpopulation of platelets.[11]

Clone

The CD23 antibody, clone EBVCS-5 (Leu 20),[7,12] is derived from the hybridization of Sp2/0 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with an in vitro transformed EBV cell line.[13]

Composition

The CD23 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following are supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	50	20	12.5	1	12.5	Gelatin	0.1% Sodium azide
PE	50	20	12.5	1	12.5	Gelatin	0.1% Sodium azide

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

bdbiosciences.com
ResearchApplications@bd.com

5/2015 23-1757-07

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
APC	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide
APC-R700[b]	100	5	6.25	0.5	12.5	BSA	ProClin® 300

a. Volume required to stain 10^6 cells.
b. BD Horizon™ APC-R700

CAUTION Some APC-R700 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

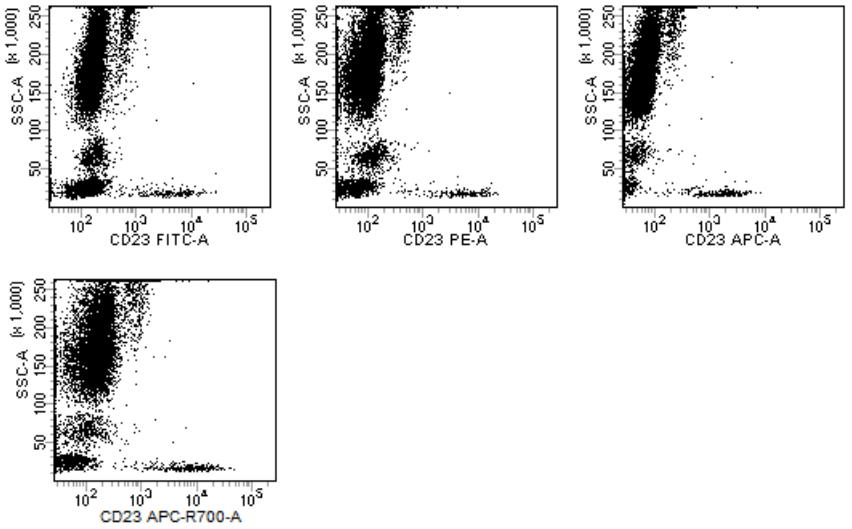
PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 488 nm, 635 nm, or 640 nm.

The APC-R700 conjugate is read off the red laser (640 nm) using a 685 longpass mirror with a 712/21 bandpass filter. Representative data analyzed with a BD FACS™ brand flow cytometer is shown in the following plots.



HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[14,15] 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.

Some reagents are bottled with ProClin 300, and contain 0.003% of a mixture of CMIT/MIT (3:1), CAS number 55965-84-9.

Warning

H317 May cause an allergic skin reaction.

Wear protective gloves/eye protection. Wear protective clothing. Avoid breathing mist/vapours/spray. If skin irritation or rash occurs: Get medical advice/attention. IF ON SKIN: Wash with plenty of water. Dispose of contents/container in accordance with local/regional/national/international regulations.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

REFERENCES

1. Sherr E, Macy E, Kimata H, Gilly M, Saxon A. Binding the low affinity Fc epsilon R on B cells suppresses ongoing human IgE synthesis. *J Immunol.* 1989;142:481-489.
2. Yukawa K, Kikutani H, Owaki H, et al. A B cell-specific differentiation antigen, CD23, is a receptor for IgE (FcεR) on lymphocytes. *J Immunol.* 1987;138:2576-2580.
3. Kwon HS, Park MC, Kim DG, et al. Identification of CD23 as a functional receptor for the proinflammatory cytokine AIMP1/p43. *J Cell Sci.* 2012;125:4620-4629.
4. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.
5. Gordon J, Webb AJ, Walker L, Guy GR, Rowe M. Evidence of an association between CD23 and the receptor for a low molecular weight B cell growth factor. *Eur J Immunol.* 1986;16:1627-1630.
6. Thorley-Lawson DA, Nadler LM, Bhan AK, Schooley RT. BLAST-2 [EBVCS], an early cell surface marker of human B cell activation, is superinduced by Epstein Barr virus. *J Immunol.* 1985;134:3007-3012.
7. Nadler LM. B Cell/Leukemia Panel Workshop: Summary and Comments. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human B Lymphocytes.* Vol 2. New York, NY: Springer-Verlag; 1986:3-43.
8. Kikutani H, Suemura M, Owaki H, et al. Fcε receptor, a specific differentiation marker transiently expressed on mature B cells before isotype switching. *J Exp Med.* 1986;164:1455-1469.
9. Kikutani H, Inui S, Sato R, et al. Molecular structure of human lymphocyte receptor for immunoglobulin E. *Cell.* 1986;47:657-665.
10. Gordon J, Rowe M, Walker L, Guy G. Ligation of the CD23,p45 (BLAST-2,EBVCS) antigen triggers the cell-cycle progression of activated B lymphocytes. *Eur J Immunol.* 1986;16:1075-1080.
11. Capron M, Jouault T, Prin L, et al. Functional study of a monoclonal antibody to IgE Fc receptor (Fc epsilon R2) of eosinophils, platelets, and macrophages. *J Exp Med.* 1986;164:72-89.
12. Sarfati M, Ishihara H, Delespesse G. CD23 Workshop Panel report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1995:530-533.
13. Kintner C, Sugden B. Identification of antigenic determinants unique to the surfaces of cells transformed by Epstein-Barr virus. *Nature.* 1981;294:458-460.
14. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Third Edition.* Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29-A3.
15. Centers for Disease Control. Perspectives in disease prevention and health promotion 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.

PATENTS AND TRADEMARKS

Monoclonal Antibodies Detecting Human Antigens

CD24 (ML5)

Form	Catalog number
APC-H7	658331

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

RESEARCH APPLICATIONS

Research applications include:

- Characterization of leukemias and lymphomas[1]
- Analysis of the regulation of B-cell differentiation and proliferation[2,3]
- Detection of granulocyte activation[4]

DESCRIPTION

Specificity

The CD24 antibody reacts specifically with a 35–45 kilodalton (kDa) highly glycosylated sialoprotein anchored to the cell surface by glycosylphosphatidylinositol (GPI).[5] The CD24 antigen is also known as heat stable antigen (HSA) homologue, BA-1, and small cell lung carcinoma cluster-4 antigen.[5,6]

Antigen distribution

The CD24 antigen is expressed on B cells and most B-cell lines, but it is not expressed on plasma cells.[7] The CD24 antigen is also expressed on neutrophils,[7] follicular dendritic cells,[8] and epithelial cells.[9] The CD24 antigen may play a role in the regulation of B-cell proliferation and activation,[10] and cross-linking of CD24 induces a calcium flux and oxidative burst in granulocytes.[11]

Clone

The CD24 antibody, clone ML5,[7] is derived from the hybridization of mouse myeloma cells isolated from the spleen of immunized mice.

Composition

The CD24 antibody is composed of mouse IgG_{2a} heavy chains and kappa light chains.

Product configuration

The following is supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)[a]	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
APC-H7	100	5	100	0.5	200	BSA	ProClin™ 300

a. Volume required to stain 10^6 cells.

PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

CAUTION Some APC-H7 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

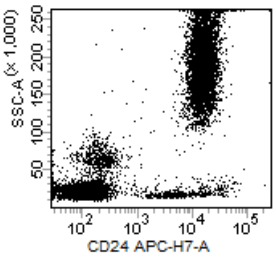
Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

bdbiosciences.com
ResearchApplications@bd.com

3/2020 23-15269-03

REPRESENTATIVE DATA

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 635 nm. Representative data analyzed with a BD FACS™ brand flow cytometer is shown in the following plot.



HANDLING AND STORAGE

Store vials at 2°C–8°C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

WARNING

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[12,13] 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.

The APC-H7 conjugate is bottled with ProClin 300, and contains 0.003% of a mixture of CMIT/MIT (3:1), CAS number 55965-84-9.

	Warning
	H317 May cause an allergic skin reaction.
	Wear protective clothing/eye protection. Wear protective gloves. Contaminated work clothing should not be allowed out of the workplace. Avoid breathing mist/vapors/spray. If skin irritation or rash occurs, get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

REFERENCES

1. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.
2. de Rie MA, Terpstra FG, van Lier RAW, Von dem Borne AEG, Miedema F. Identification of functional epitopes on Workshop-defined B-cell membrane molecules. In: McMichael AJ, Beverley PCL, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:402-405.

3. Rabinovitch PS, Clark EA, Pezzutto A, Ledbetter JA, Draves KE. Modulation of human B-cell activation with Workshop monoclonal antibodies to B-cell-associated differentiation antigens. In: McMichael AJ, Beverley PCL, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:435-439.

4. Fischer GF, Majdic O, Gadd S, Knapp W. Signal transduction in lymphocytic and myeloid cells via CD24, a new member of phosphoinositol-anchored membrane molecules. *J Immunol.* 1990;144:638-641.

5. Zola H, Swart B, Nicholson I, Voss E. *Leukocyte and Stromal Cell Molecules: The CD Markers.* Hoboken, NJ: John Wiley & Sons, Inc.; 2007.

6. Weber E, Lehmann HP, Beck-Sickinger AG, et al. Antibodies to the protein core of the small cell lung cancer workshop antigen cluster-w4 and to the leucocyte workshop antigen CD24 recognize the same short protein sequence leucine-alanine-proline. *Clin Exp Immunol.* 1993;93:279-285.

7. Le Bien TW. CD24 Workshop Panel report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1995:539-541.

8. Petrasch S, Perez-Alvarez C, Schmitz J, Kosco M, Brittinger G. Antigenic phenotyping of human follicular dendritic cells isolated from nonmalignant and malignant lymphatic tissue. *Eur J Immunol.* 1990;20:1013-1018.

9. Barclay AN, Brown MH, Law SKA, McKnight AJ, Tomlinson MG, van der Merwe PA. *The Leucocyte Antigen FactsBook*. 2nd ed. San Diego, CA: Academic Press; 1997.

10. Kay R, Rosten PM, Humphries RK. CD24, a signal transducer modulating B cell activation responses, is a very short peptide with a glycosyl phosphatidylinositol membrane anchor. *J Immunol.* 1991;147:1412-1416.

11. Lund-Johansen F, Olweus J, Symington FW, et al. Activation of human monocytes and granulocytes by monoclonal antibodies to glycosylphosphatidylinositol-anchored antigens. *Eur J Immunol.* 1993;23:2782-2791.

12. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Third Edition.* Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29-A3.

13. Centers for Disease Control. Perspectives in disease prevention and health promotion 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.

PATENTS AND TRADEMARKS

Technical Data Sheet

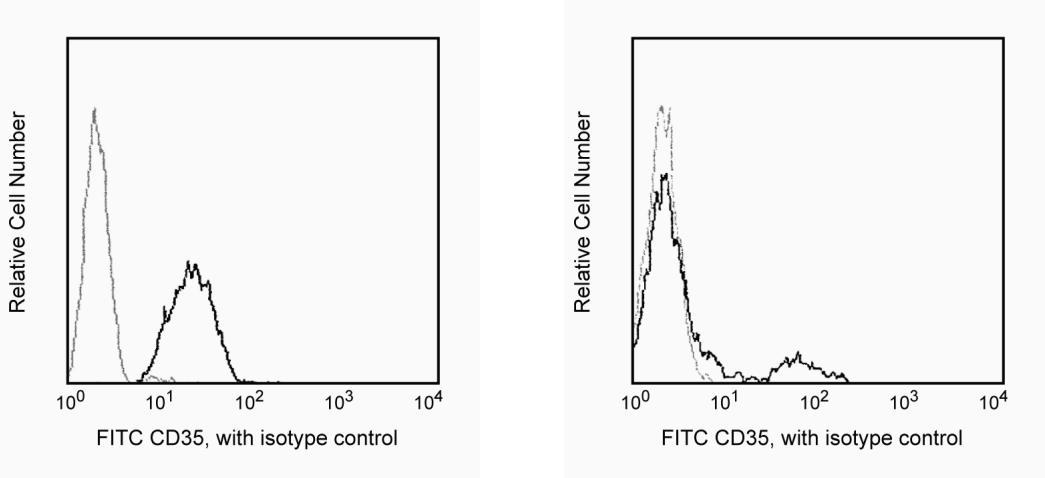
FITC Mouse Anti-Human CD35

Product Information

Material Number:	**555452**
Alternate Name:	CR1; Complement receptor type 1; C3b/C4b receptor; C3BR; C4BR; KN
Size:	100 Tests
Vol. per Test:	20 µl
Clone:	E11
Immunogen:	Human Cells of the Monocyte Lineage
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	III 204
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The E11 monoclonal antibody specifically binds to CD35. CD35 is also known as Complement receptor type 1 (CR1), C3b/C4b receptor, C3BR, C4BR, Immune adherence receptor, or KN. CD35 is a type I transmembrane glycoprotein that exists in four allelic forms of 160, 190, 220 and 250 kDa. CD35 serves as a receptor for complement fragments C3b, iC3b, C3dg, C4b, iC3, and iC4. It enhances phagocytosis by neutrophils and monocytes and regulates complement activation. It is expressed on erythrocytes, granulocytes, monocytes, B cells, and some dendritic cells, T cells, and NK cells. It binds complement components C3b and C4b, mediating. This antibody cannot inhibit the phagocytic capacity of granulocytes. The CD35 antibody is useful in studies of cells that express complement receptors.



Flow cytometric analysis of CD35 expression on human peripheral blood granulocytes (left panel) and lymphocytes (right panel). *Whole blood was stained with either FITC Mouse Anti-Human CD35 (Cat. No. 555452, solid line histogram), or FITC Mouse IgG1, κ Isotype Control (Cat. No. 555748, dashed line histogram). Erythrocytes were lysed with BD FACS™ Lysing Solution (Cat. No. 349202). Fluorescence histograms were derived from gated events with the side and forward light-scatter characteristics of viable granulocytes or lymphocytes. Flow cytometry was carried out on a BD FACScan™ System.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Flow cytometry	Routinely Tested

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.8408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**

Suggested Companion Products

Catalog Number	Name	Size	Clone
555748	FITC Mouse IgG1, κ Isotype Control	100 Tests	MOPC-21
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Barclay NA, Brown MH, Birkeland ML, et al, ed. *The Leukocyte Antigen FactsBook.* San Diego, CA: Academic Press; 1997(Biology)

Dougherty GJ, Selvendran Y, Murdoch S, Palmer DG, Hogg N. The human mononuclear phagocyte high-affinity Fc receptor, FcRI, defined by a monoclonal antibody, 10.1. *Eur J Immunol.* 1987; 17(10):1453-1459. (Biology)

Schlossman SF. Stuart F. Schlossman .. et al., ed. *Leucocyte typing V : white cell differentiation antigens : proceedings of the fifth international workshop and conference held in Boston, USA, 3-7 November, 1993.* Oxford: Oxford University Press; 1995(Biology)

BD CD36 (CLB-IVC7)

Monoclonal Antibodies Detecting Human Antigens

Form	Catalog number
FITC	656151

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include:

- Enumeration of immature erythroid cells[1,2]
- Analysis of erythroid differentiation[1-3]
- Determination of macrophage binding to oxidized LDL[4]
- Characterization of leukemias and lymphomas[5]

Description

Specificity

The CD36 antibody specifically binds to the 88-kilodalton (kDa) glycoprotein IV of platelets.[6] The antigen is also known as GPIV, GP4, GP3B, thrombospondin receptor, PASIV, FAT, and SCARB3.

Antigen distribution

The CD36 antigen is expressed on platelets, megakaryocytes, monocytes, macrophages, dendritic cells, erythroid precursors, adipocytes, and some endothelial and epithelial cells.[7,8]

The CD36 antigen is the receptor for extracellular matrix proteins such as collagen and thrombospondin.[8,9] The CD36 antigen can mediate the adhesion of erythrocytes infected with the human malaria parasite, *Plasmodium falciparum*.[10-12] The CD36 antigen functions as a scavenger receptor in macrophages.[7]

Clone

The CD36 antibody, clone CLB-IVC7,[13] is derived from the hybridization of Sp2/0 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with human monocytes.

Composition

The CD36 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following is supplied in buffer containing a stabilizer and a preservative.

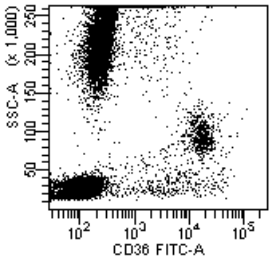
Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	50	20	12.5	1	12.5	Gelatin	0.1% Sodium azide

Procedure

Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Representative Data

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 488 nm. Representative data analyzed with a BD flow cytometer is shown in the following plot.



Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Warning

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[14,15] 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.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

References

1. Scicchitano MS, McFarland DC, Tierney LA, Narayanan PK, Schwartz LW. In vitro expansion of human cord blood CD36+ erythroid progenitors: temporal changes in gene and protein expression. *Exp Hematol.* 2003;31:760-769.

2. Garcon L, Lacout C, Svinartchouk F, et al. Gfi-1B plays a critical role in terminal differentiation of normal and transformed erythroid progenitor cells. *Blood.* 2005;105:1448-1455.

3. de Wolf JT, Muller EW, Hendriks DH, Halie RM, Vellenga E. Mast cell growth factor modulates CD36 antigen expression on erythroid progenitors from human bone marrow and peripheral blood associated with ongoing differentiation. *Blood.* 1994;84:59-64.

4. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. CD36 is a receptor for oxidized low density lipoprotein. *J Biol Chem.* 1993;268:11811-11816.

5. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.

6. Zola H, Swart B, Nicholson I, Voss E. *Leukocyte and Stromal Cell Molecules: The CD Markers.* Hoboken, NJ: John Wiley & Sons, Inc.; 2007.

7. Barclay AN, Brown MH, Law SKA, McKnight AJ, Tomlinson MG, van der Merwe PA. *The Leucocyte Antigen FactsBook.* 2nd ed. San Diego: Academic Press; 1997.

8. Greenwalt DE, Lipsky RH, Ockenhouse CF, Ikeda H, Tandon NN, Jamieson GA. Membrane glycoprotein CD36: a review of its roles in adherence, signal transduction, and transfusion medicine. *Blood.* 1992;80:1105-1115.

9. Tandon NN, Kralisz U, Jamieson GA. Identification of glycoprotein IV (CD36) as a primary receptor for platelet-collagen adhesion. *J Biol Chem.* 1989;264:7576-7583.

10. Handunnetti SM, van Schravendijk MR, Hasler T, Barnwell JW, Greenwalt DE, Howard RJ. Involvement of CD36 on erythrocytes as a rosetting receptor for Plasmodium falciparum-infected erythrocytes. *Blood.* 1992;80:2097-2104.

11. Ockenhouse CF, Magowan C, Chulay JD. Activation of monocytes and platelets by monoclonal antibodies or malaria-infected erythrocytes binding to the CD36 surface receptor in vitro. *J Clin Invest.* 1989;84:468-475.

12. Ockenhouse CF, Tandon NN, Magowan C, Jamieson GA, Chulay JD. Identification of a platelet membrane glycoprotein as a falciparum malaria sequestration receptor. *Science.* 1989;243:1469-1471.

13. de Haas M, von dem Borne AEG. CD36 Workshop Panel report. In: Kishimoto T, Kikutani H, von dem Borne AEG, et al., eds. *Leucocyte Typing VI: White Cell Differentiation Antigens.* New York, NY: Garland Publishing, Inc.; 1997:636-637.

14. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.

15. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

Patents and Trademarks

For US patents that may apply, see bd.com/patents.

Contact Information

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

bdbiosciences.com
ResearchApplications@bd.com

BD CD38 (HB7)

Monoclonal Antibodies Detecting Human Antigens

Form	Catalog number	Form	Catalog number
Pure	347680	APC	340439
FITC	340927	APC-H7	656646
PE	347687	APC-R700	659127
PE-Cy7	335790	V450	646851

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include:

- Studies of T-lymphocyte activation[1–3]
- Examination of B-lymphocyte differentiation[1,3]
- Research on human immunodeficiency[1,4–7]
- Investigation into leukemia cells[1,2,8,9]

Description

Specificity

The CD38 antibody recognizes an integral membrane glycoprotein of 45 kilodaltons (kDa), with a protein core of 35 kDa.[10] The CD38 antigen is also known as T10, ADP-ribosyl cyclase, and cyclic ADP ribose hydrolase 1.

Antigen distribution

The CD38 antigen is expressed on essentially all pre-B lymphocytes, plasma cells, and thymocytes.[10] It is also present on activated T lymphocytes, natural killer (NK) lymphocytes, myeloblasts, and erythroblasts.[3-5,10–13] The antigen is expressed during the early stages of T- and B-lymphocyte differentiation, is lost during the intermediate stages of maturation, and then reappears during the final stages of maturation.[3,6,7,10] The CD38 antigen is expressed on 90% of $CD34^+$ cells, and is not expressed on pluripotent stem cells. Coexpression of CD38 antigen on $CD34^+$ cells indicates lineage commitment of those cells.[13,14] It is also expressed in T- and B-acute lymphoblastic leukemia (ALL), Burkitt's lymphoma, multiple myeloma, acute myeloid leukemia (AML),[2,9] and chronic lymphocytic leukemia (CLL).[8]

The CD38 antigen acts as a bifunctional ectoenzyme that catalyzes both the synthesis and the hydrolysis of a Ca^{++} mobilizing agent, cyclic ADP-ribose.[15] This intracellular calcium plays an important role in cell signaling pathways. The CD38 antigen is a counter-receptor for CD31, playing a role in adhesion of lymphocytes to endothelial cells.[16] The CD38 antigen participates in signal transduction through activation of the Syk and Bruton protein kinases,[17,18] leading to cell growth, apoptosis, and differentiation.[15]

Clone

The CD38 antibody, clone HB7,[19] is derived from the hybridization of P3-X63-Ag8.653 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with the BJAB cell line.[3]

Composition

The CD38 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following are supplied in phosphate buffered saline (PBS) containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
Pure	100	20	50	2.0	25	Gelatin	0.1% Sodium azide
FITC	50	20	6	1.0	6	Gelatin	0.1% Sodium azide
PE	100	20	25	2.0	12.5	Gelatin	0.1% Sodium azide
PE-Cy7	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide
APC	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide
APC-H7	100	5	25	0.5	50	BSA	CMIT/MIT (3:1)
APC-R700[a]	100	5	12.5	0.5	25	BSA	CMIT/MIT (3:1)
V450[a]	100	5	12.5	0.5	25	Gelatin	0.1% Sodium azide

[a] BD Horizon™ APC-R700, BD Horizon™ V450

CAUTION Higher levels of nonspecific staining can result when ammonium chloride lysis is used for cell preparation before staining.

CAUTION Some PE-Cy7, APC-H7, and APC-R700 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

CAUTION Prolonged exposure of cells to paraformaldehyde can lead to increased autofluorescence in the violet channels. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

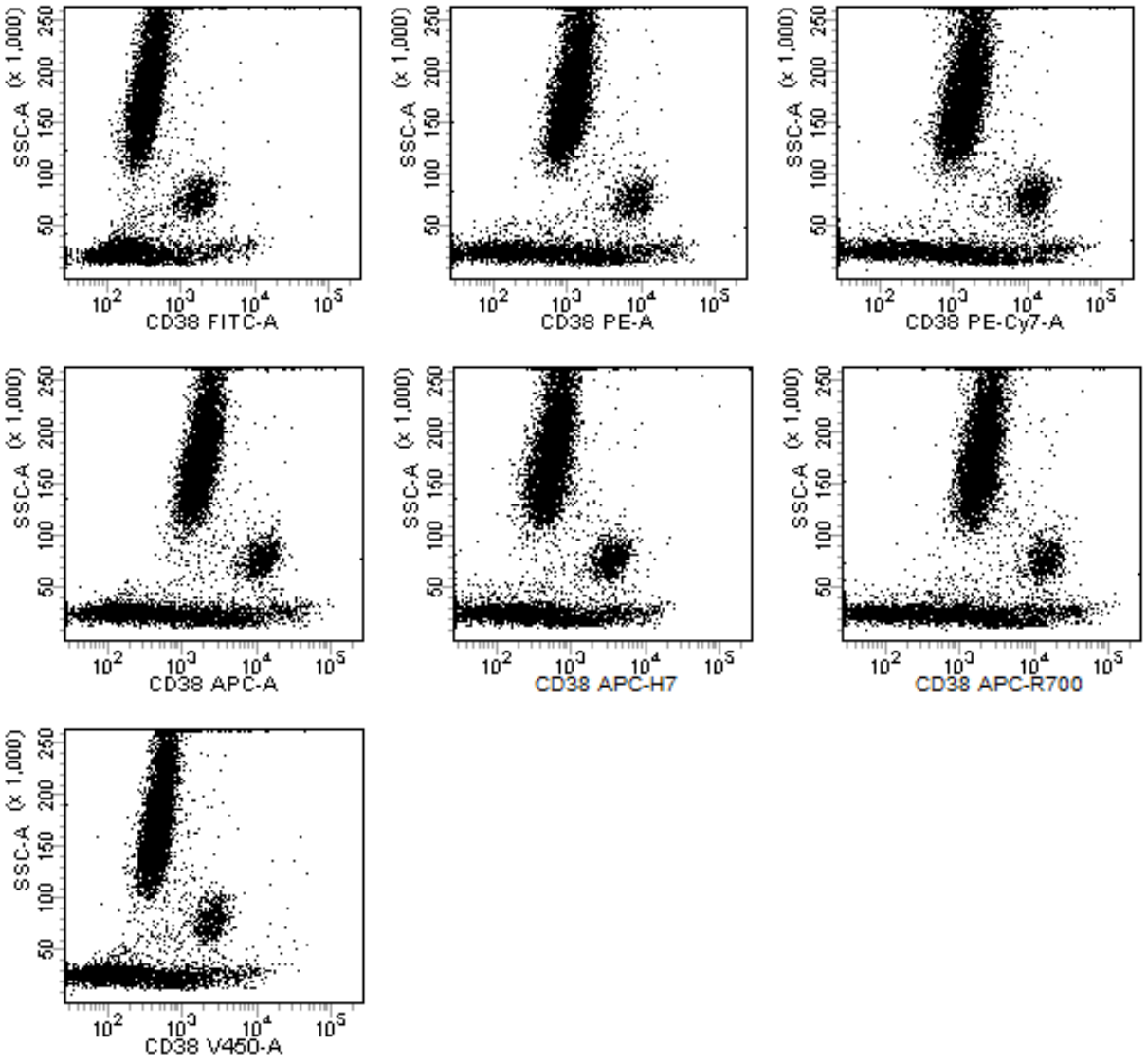
Procedure

Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Representative Data

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 405 nm, 488 nm, 635 nm, or 640 nm.

The APC-R700 conjugate is read off the red laser (640 nm) using a 685 longpass mirror with a 712/21 bandpass filter. Representative data analyzed with a BD flow cytometer is shown in the following plots.



Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Warning

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[20,21] 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.

The APC-H7 conjugate contains 0.00265% of a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one [CMIT/MIT (3:1)], CAS number 55965-84-9). The APC-R700 conjugate contains of a mixture of 0.0028% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one [CMIT/MIT (3:1)], CAS number 55965-84-9. These reagents are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

	Warning
	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P273: Avoid release to the environment.
Response	P302+P352: IF ON SKIN: Wash with plenty of water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent.

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.

References

1. Deaglio S, Mehta K, Malavasi F. Human CD38: a (r)evolutionary story of enzymes and receptors. *Leuk Res.* 2001;25:1-12.
2. Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. *Proc Natl Acad Sci USA.* 1980;77:1588-1592.

3. Tedder TF, Clement LT, Cooper MD. Discontinuous expression of a membrane antigen (HB-7) during B lymphocyte differentiation. *Tissue Antigens.* 1984;24:140-149.

4. Giorgi JV. Lymphocyte subset measurements: significance in clinical medicine. In: Rose NR, Friedman H, Fahey JL, eds. *Manual of Clinical Laboratory Immunology.* 3rd ed. Washington, DC: American Society for Microbiology; 1986:236-246.

5. Nicholson JKA, Jones BM. Immunophenotyping by flow cytometry: its use in HIV infection. *Labmedica.* 1989;6:21-26.

6. Salazar-Gonzalez JF, Moody DJ, Giorgi JV, Martinez-Maza O, Mitsuyasu RT, Fahey JL. Reduced ecto-5'-nucleotidase activity and enhanced OKT10 and HLA-DR expression on CD8 (T suppressor/cytotoxic) lymphocytes in the acquired immune deficiency syndrome: evidence of CD8 cell immaturity. *J Immunol.* 1985;135:1778-1785.

7. Tedder TF, Crain MJ, Kubagawa H, Clement LT, Cooper MD. Evaluation of lymphocyte differentiation in primary and secondary immunodeficiency diseases. *J Immunol.* 1985;135:1786-1791.

8. Ghia P, Guida G, Stella S, et al. The pattern of CD38 expression defines a distinct subset of chronic lymphocytic leukemia (CLL) patients at risk of disease progression. *Blood.* 2003;101:1262-1269.

9. Pezzutto A, Behm F, Callard RE, et al. Flow cytometry analysis of the B-cell blind panel: joint report. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:165-174.

10. Dörken B, Möller P, Pezzutto A, Schwartz-Albiez R, Moldenhauer G. B-cell antigens: CD38. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:86.

11. Landay A, Ohlsson-Wilhelm B, Giorgi JV. Application of flow cytometry to the study of HIV infection. *AIDS.* 1990;4:479-497.

12. Terstappen LW, Hollander Z, Meiners H, Loken MR. Quantitative comparison of myeloid antigens on five lineages of mature peripheral blood cells. *J Leukoc Biol.* 1990;48:138-148.

13. Terstappen LW, Huang S, Safford M, Lansdorp PM, Loken MR. Sequential generations of hematopoietic colonies derived from single nonlineage-committed $CD34^{+}CD38^{-}$ progenitor cells. *Blood.* 1991;77:1218-1227.

14. Terstappen LWMM, Huang S, Picker LJ. Flow cytometric assessment of human T-cell differentiation in thymus and bone marrow. *Blood.* 1992;79:666-677.

15. Mehta K, Shahid U, Malavasi F. Human CD38, a cell-surface protein with multiple functions. *FASEB J.* 1996;10:1408-1417.

16. Deaglio S, Morra M, Mallone R, et al. Human CD38 (ADP-ribosyl cyclase) is a counter-receptor of CD31, an Ig superfamily member. *J Immunol.* 1998;160:395-402.

17. Kikuchi Y, Yasue T, Miyake K, Kimoto M, Takatsu K. CD38 ligation induces tyrosine phosphorylation of Bruton tyrosine kinase and enhanced expression of interleukin 5-receptor alpha chain: synergistic effects with interleukin 5. *Proc Natl Acad Sci USA.* 1995;92:11814-11818.

18. Silvennoinen O, Nishigaki H, Kitanaka A, et al. CD38 signal transduction in human B cell precursors. Rapid induction of tyrosine phosphorylation, activation of syk tyrosine kinase, and phosphorylation of phospholipase C-gamma and phosphatidylinositol 3-kinase. *J Immunol.* 1996;156:100-107.

19. Ling NR, Maclennan ICM, Mason DY. B-cell and plasma cell antigens: new and previously defined clusters. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:302-335.

20. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.

21. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

Patents and Trademarks

For US patents that may apply, see bd.com/patents.

Cy™ is a trademark of GE Healthcare. This product is subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and is made and sold under license from GE Healthcare. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one, return this material, unopened, to BD Biosciences, 155 North McCarthy Boulevard, Milpitas, California 95035, and any money paid for the material will be refunded.

Contact Information

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

bdbiosciences.com
ResearchApplications@bd.com

BD CD43 (1G10)

Monoclonal Antibodies Detecting Human Antigens

Form	Catalog number
APC-H7	655407

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include:

- Research on T cell-macrophage interactions[1]
- Research on neutrophil spreading and movement[2,3]
- Study of *Streptococcus gordonii* DL1 binding to myeloid cells[4]

Description

Specificity

The CD43 antibody reacts specifically with the major 95–135-kilodalton (kDa) sialoglycoprotein found on most human leucocytes.[5] The CD43 antigen is also known as leukosialin or sialophorin.

Antigen distribution

The CD43 antigen is expressed on T cells, natural killer (NK) cells, pre-B and activated B cells,[5] granulocytes,[6] and neutrophils.[3] The CD43 antigen is differentially expressed on subpopulations of mononuclear phagocytic cells.[7] It is not present on most peripheral blood resting B cells, erythrocytes, and non-hematopoietic cells.[5] Additionally, expression of the CD43 antigen by committed myeloid, erythroid, and lymphoid progenitors has been reported.

The CD43 antigen may be involved in the regulation of B, T, and NK cell function.[5,8–10] The CD43 antigen plays a role in cell adhesion, which can be positive or negative, depending on the context.[2,11] The CD43 antigen serves as a counter-receptor for CD169, the prototypical member of the sialic acid binding Ig-like lectins (Siglec) family expressed on macrophages.[1]

Clone

The CD43 antibody, clone 1G10,[5] is derived from the hybridization of X63 mouse myeloma cells with spleen cells isolated from mice immunized with a lymph node suspension from a patient with Hodgkin lymphoma.

Composition

The CD43 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following is supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
APC-H7	100	5	25	0.5	50	BSA	CMIT/MIT (3:1)

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

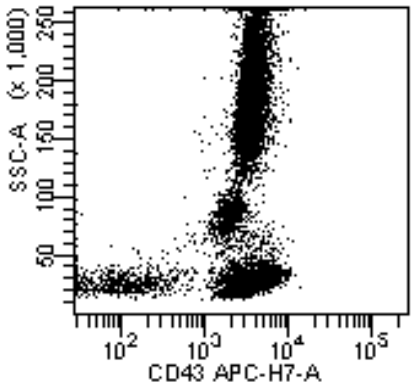
CAUTION Some APC-H7 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

Procedure

Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Representative Data

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 635 nm. Representative data analyzed with a BD flow cytometer is shown in the following plot.



Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Warning

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[12,13] 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.

The APC-H7 conjugate contains a mixture of 0.00236% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one [CMIT/MIT (3:1)], CAS number 55965-84-9. These reagents are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

	Warning
	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P273: Avoid release to the environment.

	Warning
Response	P302+P352: IF ON SKIN: Wash with plenty of water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

References

1. van den Berg TK, Nath D, Ziltener HJ, et al. Cutting edge: CD43 functions as a T cell counterreceptor for the macrophage adhesion receptor sialoadhesin (Siglec-1). *J Immunol.* 2001;166:3637-3640.
2. Matsumoto M, Shigeta A, Miyasaka M, Hirata T. CD43 plays both antiadhesive and proadhesive roles in neutrophil rolling in a context-dependent manner. *J Immunol.* 2008;181:3628-3635.
3. Nathan C, Xie QW, Halbwachs-Mecarelli L, Jin WW. Albumin inhibits neutrophil spreading and hydrogen peroxide release by blocking the shedding of CD43 (sialophorin, leukosialin). *J Cell Biol.* 1993;122:243-256.
4. Urano-Tashiro Y, Yajima A, Takashima E, Takahashi Y, Konishi K. Binding of the Streptococcus gordonii DL1 surface protein Hsa to the host cell membrane glycoproteins CD11b, CD43, and CD50. *Infect Immun.* 2008;76:4686-4691.
5. Horejsi V, Stockinger H. CD43 Workshop Panel report. In: Kishimoto H, Kikutani H, von dem Borne AEGK, et al, eds. *Leucocyte Typing VI: White Cell Differentiation Antigens.* New York: Garland Publishing, Inc.; 1998:494-497.
6. Bazil V, Strominger JL. CD43, the major sialoglycoprotein of human leukocytes, is proteolytically cleaved from the surface of stimulated lymphocytes and granulocytes. *Proc Natl Acad Sci U S A.* 1993;90:3792-3796.
7. Howell DN, Ahuja V, Jones L, Blow O, Sanfilippo FP. Differential expression of CD43 (leukosialin, sialophorin) by mononuclear phagocyte populations. *J Leukoc Biol.* 1994;55:536-544.
8. Bettaieb A, Farace F, Mitjavila MT, et al. Use of a monoclonal antibody (GA3) to demonstrate lineage restricted O-glycosylation on leukosialin during terminal erythroid differentiation. *Blood.* 1988;71:1226-1233.

9. Borche L, Lozano F, Vilella R, Vives J. CD43 monoclonal antibodies recognize the large sialoglycoprotein of human leukocytes. *Eur J Immunol.* 1987;17:1523-1526.

10. Zola H, Swart B, Nicholson I, Voss E. *Leukocyte and Stromal Cell Molecules: The CD Markers.* Hoboken, NJ: John Wiley & Sons, Inc.; 2007.

11. Ardman B, Sikorski MA, Staunton DE. CD43 interferes with T-lymphocyte adhesion. *Proc Natl Acad Sci U S A.* 1992;89:5001-5005.

12. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.

13. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

Patents and Trademarks

For US patents that may apply, see bd.com/patents.

Contact Information

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

bdbiosciences.com
ResearchApplications@bd.com

BD CD64 (10.1)

Monoclonal Antibodies Detecting Human Antigens

Form	Catalog number
PE	644385

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include studies of:

- Inflammatory response[1]
- Neutrophil and monocyte function[1, 2]
- Dendritic cell function[3]
- Sepsis[4]
- Vaccine development[5]
- Cancer therapy[6]

Description

Specificity

The CD64 antibody recognizes the 72-kilodalton (kDa) human FcγRI that can bind monomeric IgG.[1,2]

Antigen distribution

The CD64 antigen is one of three Fc receptors for immunoglobulins, including human FcγRII (CD32 antigen) and human FcγRIII (CD16 antigen), present on the surface of leucocytes.[1,2] While FcγRII and FcγRIII are low-affinity receptors for immunoglobulin, FcγRI bind with high affinity.[1,2] Structurally, the CD64 antigen possesses an extracellular region of 292 amino acids with three C2 set Ig-like domains, a 21-amino acid transmembrane region, and a charged cytoplasmic tail of 61 amino acids.[1,2] Stable expression of FcγRI requires coexpression of the IgG-binding α-chain as an oligomeric complex with the FcR γ-chain homodimer.[7]

CD64, a key receptor in the development of immune responses, has a dual role as a low-affinity receptor for IgG_3 and a high-affinity receptor for IgG_{2a} linking innate and adaptive immunities.

The CD64 antigen is expressed on monocytes, macrophages, at low levels on polymorphonuclear neutrophils (PMNs),[1,2] and on a subpopulation of circulating dendritic cells.[3] CD64 is an early granulomonocytic lineage marker on $CD34^+$ hematopoietic progenitors.[8] Soluble human FcγRI molecules have been found in human serum.[9] Three genes have been characterized for FcγRI, each gene consisting of six exons, spanning 9.5 kilobases, and localized to chromosome 1.[9,10]

Clone

The CD64 antibody, clone 10.1, is derived from a fusion of Sp2/0-Ag14 cells with spleen cells from a BALB/c mouse that was immunized first with 2×10^7 rheumatoid synovial fluid cells and on subsequent occasions with 1.5×10^7 fibronectin-purified human monocytes obtained from pools of blood group–matched donors.[11]

Composition

The CD64 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following reagent is supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
PE	50	20	25	1.0	25	BSA	0.1% Sodium azide

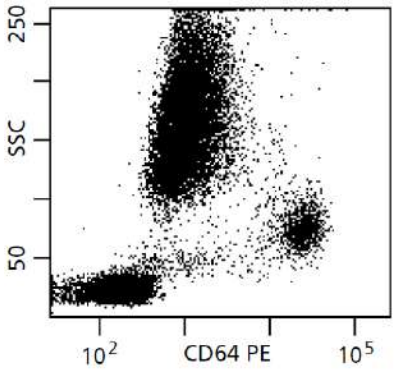
Procedure

Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Representative Data

Flow cytometric analysis was performed on whole blood. Laser excitation was at 488 nm.

Figure 1 Representative data analyzed with a BD flow cytometer



Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Warning

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[12,13] 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.

	Warning
	H317: May cause an allergic skin reaction.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection.

	Warning
Response	P302+P352: IF ON SKIN: Wash with plenty of water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P321: Specific treatment (see supplemental first aid instructions on Safety Data Sheet). P321: Specific treatment (see Safety Data Sheet). P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

References

1. van de Winkel JG, Capel PJ. Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunol Today*. 1993;14:215-221.
2. van de Winkel JG, Anderson CL. Biology of human immunoglobulin G Fc receptors. *J Leukoc Biol*. 1991;49:511-524.
3. Fanger NA, Wardwell K, Shen L, Tedder TF, Guyre PM. Type I (CD64) and type II (CD32) Fc gamma receptor-mediated phagocytosis by human blood dendritic cells. *J Immunol*. 1996;157:541-548.
4. Davis BH, Bigelow NC, Curnutte JT, Ornvold K. Neutrophil CD64 expression: potential diagnostic indicator of acute inflammation and therapeutic monitor of interferon-γ therapy. *Lab Hematol*. 1995;1:3-12.
5. Gosselin EJ, Wardwell K, Gosselin DR, Alter N, Fisher JL, Guyre PM. Enhanced antigen presentation using human Fc gamma receptor (monocyte/macrophage)–specific immunogens. *J Immunol*. 1992;149:3477-3481.
6. Valerius T, Repp R, de Wit TP, et al. Involvement of the high-affinity receptor for IgG (Fc gamma RI; CD64) in enhanced tumor cell cytotoxicity of neutrophils during granulocyte colony-stimulating factor therapy. *Blood*. 1993;82:931-939.
7. van Vugt MJ, Heijnen IAFM, Capel PJ, et al. FcR gamma-chain is essential for both surface expression and function of human Fc gamma RI (CD64) in vivo. *Blood*. 1996;87:3593-3599.
8. Olweus J, Terstappen LWMM, Thompson PA, Lund-Johansen F. Expression and function of receptors for stem cell factor and erythropoietin during lineage commitment of human hematopoietic progenitor cells.

Blood. 1996;88:1594-1607.

9. Ernst LK, van de Winkel JG, Chiu IM, Anderson CL. Three genes for the human high affinity Fc receptor for IgG (Fc gamma RI) encode four distinct transcription products. *J Biol Chem*. 1992;267:15692-15700.
10. van de Winkel JG, Ernst LK, Anderson CL, Chiu IM. Gene organization of the human high affinity receptor for IgG, Fc gamma RI (CD64). Characterization and evidence for a second gene. *J Biol Chem*. 1991;266:13449-13455.
11. Dougherty GJ, Selvendran Y, Murdoch S, Palmer DG, Hogg N. The human mononuclear phagocyte high-affinity Fc receptor, FcRI, defined by a monoclonal antibody, 10.1. *Eur J Immunol*. 1987;17:1453-1459.
12. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
13. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

Patents and Trademarks

For US patents that may apply, see bd.com/patents.

Contact Information

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

bdbiosciences.com
ResearchApplications@bd.com

BD CD79b (SN8)

Monoclonal Antibodies Detecting Human Antigens

Form	Catalog number
FITC	335798
PE	335799
PerCP-Cy5.5	656644
APC	335800
APC-R700	657702

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include:

- Characterization of leukemias and lymphomas[1-3]
- Analysis of lymphocyte development[4]

Description

Specificity

The CD79b antibody recognizes an epitope on the extracellular domain of a 36–40 kilodalton (kDa) type I membrane glycoprotein.[5] Immunoglobulin (Ig) antigen receptors are composed of a non-covalently associated complex of Ig and two other proteins, Igα and Igβ, which have been designated as CD79a and CD79b, respectively.[5]

Antigen distribution

The CD79b antigen is expressed on surface-Ig (sIg)–positive lymphocytes and B-cell lines. It can also be found in the cytoplasm of sIg-negative cells, including most terminal deoxynucleotidyl transferase (TdT)-positive early pre-B and all cytoplasmic μ-positive pre-B–cell lines.[5] The vast majority of chronic lymphocytic leukemia cells are $CD79b^-$ while cells from other B-cell disorders usually express high levels of the CD79b antigen.[6]

Clone

The CD79b antibody, clone SN8 (3A2-2E7-1F5),[5] is derived from the hybridization of NS-1 mouse myeloma cells with spleen cells isolated from BALB/c mice immunized with cell membrane preparations from B-prolymphocytic leukemia (B-PLL) cells.[7]

Composition

The CD79b antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following are supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	50	20	25	1.0	25	Gelatin	0.1% Sodium azide
PE	50	20	25	1.0	25	Gelatin	0.1% Sodium azide
PerCP-Cy5.5	50	20	13	1.0	13	Gelatin	0.1% Sodium azide
APC	100	5	50	0.5	100	Gelatin	0.1% Sodium azide
APC-R700[a]	100	5	25	0.5	50	BSA	CMIT/MIT (3:1)

[a] BD Horizon™ APC-R700

CAUTION Some APC-R700 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

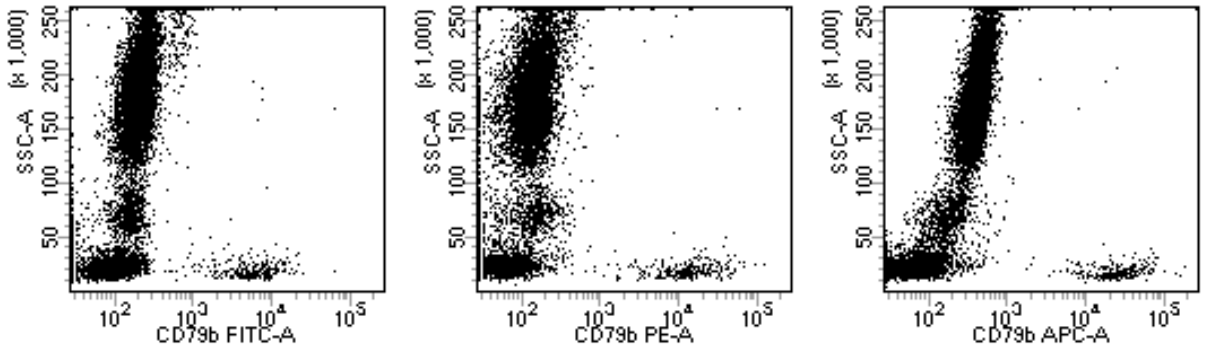
Procedure

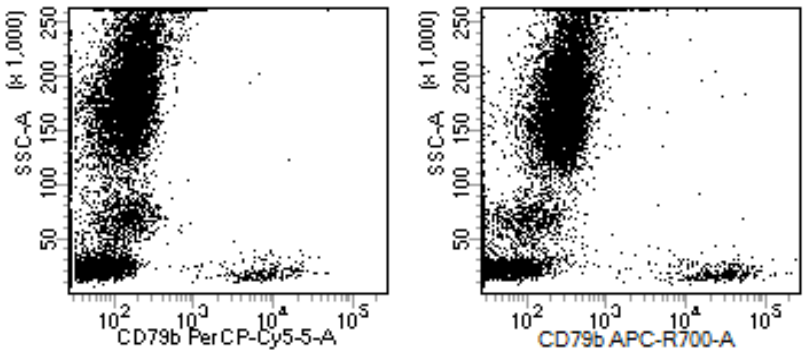
Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Representative Data

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody and gated on lymphocytes. Laser excitation was at 488 nm, 635 nm, or 640 nm.

The APC-R700 conjugate is read off the red laser (640 nm) using a 685 longpass mirror with a 712/21 bandpass filter. Representative data analyzed with a BD flow cytometer is shown in the following plots.





Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

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.

The APC-R700 conjugate contains a mixture of 0.00279% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one [CMIT/MIT (3:1)], CAS number 55965-84-9, 5-chloro-2-methyl-4-isothiazolin-3-one [EC number 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC number 220-239-6] (3:1). These reagents are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

	Warning
	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P273: Avoid release to the environment.
Response	P302+P352: IF ON SKIN: Wash with plenty of water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent.

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.

References

1. Garcia Vela J, Delgado I, Benito L, et al. CD79b expression in B cell chronic lymphocytic leukemia: its implication for minimal residual disease detection. *Leukemia.* 1999;13:1501-1505.
2. Rawstron AC, Kennedy B, Evans PA, et al. Quantitation of minimal disease levels in chronic lymphocytic leukemia using a sensitive flow cytometric assay improves the prediction of outcome and can be used to optimize therapy. *Blood.* 2001;98:29-35.
3. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.
4. Nakamura T, Kubagawa H, Cooper MD. Heterogeneity of immunoglobulin-associated molecules on human B cells identified by monoclonal antibodies. *Proc Natl Acad Sci USA.* 1992;89:8522-8526.
5. Engel P, Wagner N, Tedder TF. CD79 Workshop report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1995:667-670.
6. Zomas AP, Matutes E, Morilla R, Owusu-Ankomah K, Seon BK, Catovsky D. Expression of the immunoglobulin-associated protein B29 in B cell disorders with the monoclonal antibody SN8 (CD79b). *Leukemia.* 1996;10:1966-1970.
7. Okazaki M, Luo Y, Han T, Yoshida M, Seon BK. Three new monoclonal antibodies that define a unique antigen associated with prolymphocytic leukemia/non-Hodgkin's lymphoma and are effectively internalized after binding to the cell surface antigen. *Blood.* 1993;81:84-94.
8. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
9. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

Patents and Trademarks

For US patents that may apply, see bd.com/patents.

Cy™ is a trademark of GE Healthcare. This product is subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and is made and sold under license from GE Healthcare. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one, return this material, unopened, to BD Biosciences, 155 North McCarthy Boulevard, Milpitas, California 95035, and any money paid for the material will be refunded.

Contact Information

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

bdbiosciences.com
ResearchApplications@bd.com

BD OneFlow™ Setup Beads

25 tests per kit—Catalog No. 658620

7/2014 23-15758-00

IVD

CE

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

EC REP

Benex Limited
Pottery Road, Dun Laoghaire,
Co. Dublin, Ireland
Tel +353.1.202.5222
Fax +353.1.202.5388

BD Biosciences
European Customer Support
Tel +32.2.400.98.95
Fax +32.2.401.70.94
help.biosciences@europe.bd.com

Becton Dickinson Pty Ltd,
4 Research Park Drive,
Macquarie University Research Park,
North Ryde NSW 2113, Australia

Becton Dickinson Limited,
8 Pacific Rise, Mt. Wellington,
Auckland, New Zealand

bdbiosciences.com
ClinicalApplications@bd.com

1. INTENDED USE

BD OneFlow™ Setup beads are intended to set voltages appropriate for the BD multicolor tube assay when used with a suitably equipped BD™ flow cytometer and software designated for in vitro diagnostic use.

2. SUMMARY AND EXPLANATION

BD OneFlow Setup beads are fluorescent particles that are used to set cytometer detector photomultiplier tube voltages (PMTVs) for the BD multicolor tube assay. PMTVs are manually adjusted to place the BD OneFlow Setup beads at their lot specific median fluorescence intensity (MFI) target ranges for all fluorescence parameters. Lysed washed blood (LWB) is used to set cytometer FSC and SSC voltages to a target value range. The detector settings are then saved as Application Settings.

3. PRINCIPLES OF THE PROCEDURE

BD has developed a suite of beads that are used with BD FACSDiva™ software to standardize setup of the BD FACSCanto™ II flow cytometer with a 3-laser, 8-color 4-2H-2V BD default (4-2H-2V) optical configuration. First, BD FACSDiva™ CS&T IVD beads (CS&T IVD beads) are used to perform daily cytometer quality control. BD OneFlow Setup beads and LWB are then used to set assay-specific PMTVs and to generate Application Settings. Finally, BD™ FC beads 8-color kit for BD OneFlow™ assays (BD FC beads) is used to calculate compensation.

4. STORAGE AND HANDLING

- Store the vial at 2°C–8°C. The vial should not be frozen. Protect from exposure to light. The beads are stable until the expiration date shown on the vial label when stored as directed. Do not use after the expiration date. Do not mix the contents of one kit with another. Target values can vary between lots and this could result in inaccurate detector settings.
- After dilution, the beads are stable for
  - 1 hour at 18°C–25°C
  - 8 hours at 2°C–8°C

  WARNING Protect the diluted bead suspension from light. Some of the dyes used to manufacture the beads are very light sensitive. Fluorescence levels can change if the beads are exposed to direct light for longer than 20 minutes.

5. REAGENTS AND MATERIALS

Reagents provided

- One vial of BD OneFlow Setup beads, sufficient for 25 tests

  BD OneFlow Setup beads are supplied in phosphate buffered saline (PBS) with bovine serum albumin (BSA) and 0.1% sodium azide.
- Monthly MFI target range card

  The monthly MFI target range card contains MFI ranges for all fluorescence detectors.
- Daily MFI target range card

  The daily MFI target range card contains MFI ranges for all fluorescence detectors that are optimized for optional daily monitoring. See the *Instrument Setup Guide for BD OneFlow Assays* for more information.

Reagents and materials required but not provided

- Installer CD with OneFlow Setup template (Catalog No. 659305)

  The template contains two global worksheets (*BD OneFlow™ TMFI Setup* and *BD OneFlow™ Scatter Setup*). Be sure to order this CD prior to using the BD OneFlow Setup beads for the first time.
- Vortex mixer
- Pasteur pipets
- Micropipettor with tips
- 12 x 75-mm capped polystyrene tubes
- BD FACS Flow™ sheath fluid (Catalog No. 342003)
- BD FACSCanto II flow cytometer with a 4-2H-2V optical configuration

  See the cytometer user's guide for information.
- BD FACSDiva software v8.0.1 or later

  See the *BD FACSDiva Software Reference Manual*.
- BD FACSDiva CS&T IVD beads (Catalog No. 656046 or 656047)

  See the *BD FACSDiva CS&T IVD Beads* IFU.
- Lysed washed blood (LWB) specimen from a normal donor

  Use the blood specimen within 24 hours of collection. See Lysing the blood specimen for instructions.

- BD FACS™ lysing solution (Catalog No. 349202)

  For dilution instructions and warnings, see the reagent IFU.

- Wash buffer (filtered PBS with 0.5% BSA and 0.09% sodium azide)

Precautions

- For in vitro diagnostic use.
- Do not use BD OneFlow Setup beads beyond their expiration date or beyond the day-of-use stability period after dilution, as described in the Storage and Handling section. Beads used beyond their stability period begin to lose fluorescence, which may result in inaccurate PMTV setup.
- MFI target ranges provided on the monthly MFI target range card are bead lot specific. Verify that the bead lot number on the monthly MFI target range card matches the lot ID of the BD OneFlow Setup beads that you are using. A mismatch will result in inaccurate PMTVs and Application Settings.

6. PROCEDURE

Generate new Application Settings using BD OneFlow Setup beads and LWB at the following times:

- Once a month to ensure consistent and accurate assay-specific PMTV setup
- Each time a new lot of BD OneFlow Setup beads is used
- Each time a new lot of CS&T IVD beads is used
- Whenever a new baseline is defined using CS&T IVD beads
- After cytometer maintenance or service is performed

Installing the OneFlow Setup template

1. Insert the installer CD into the CD drive and click the installer icon.
2. Follow the prompts to install the template.

   The installer will copy and paste the template into the folder: D:\BDExport\Templates\Panel\BDPanels.

Lysing the blood specimen

You will use a LWB specimen to adjust FSC and SSC voltages.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[1,2] 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.

1. Add 100 µL of whole blood from a normal donor to a tube labeled *LWB*.
2. Add 2 mL of 1X BD FACS lysing solution.
3. Vortex 3–5 seconds to mix well.
4. Incubate for 10 minutes at 18°C–25°C.
5. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
6. Remove the supernatant without disturbing the cell pellet and leave approximately 50 µL of residual liquid in the tube.
7. Vortex 3–5 seconds to resuspend the cell pellet.

8. Add 2 mL of wash buffer to the tube.
9. Vortex 3–5 seconds to mix well.
10. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
11. Remove the supernatant without disturbing the cell pellet and leave approximately 50 µL of residual liquid in the tube.
12. Vortex 3–5 seconds to resuspend the cell pellet.
13. Add 250 µL of wash buffer to the tube.
14. Vortex 3–5 seconds to mix well.
15. Save the LWB sample to adjust FSC and SSC voltages. See Adjusting FSC and SSC on page 6.

    Store at 2°C–25°C until acquisition.

Preparing BD OneFlow Setup beads

Before preparing BD OneFlow Setup beads, verify that the CS&T IVD beads daily performance check for the 4-2H-2V configuration was completed today and passed.

1. Label a 12 x 75-mm capped polystyrene tube *Setup beads*.
2. Thoroughly mix the BD OneFlow Setup beads vial.
3. Prepare the diluted beads according to Table 1 and the task you are performing.

Table 1 BD OneFlow Setup beads preparation

Task	BD FACSFlow sheath fluid (µL)	Beads (number of drops)
First time setup	700	2
Monthly setup	350	1

4. Return the BD OneFlow Setup beads to 2°C–8°C storage.
5. Vortex the tube gently before use.

   If not acquiring immediately, store the diluted beads, protected from light, for up to:

   - 1 hour at 18°C–25°C
   - 8 hours at 2°C–8°C

Setting up the software

1. In the BD FACSDiva workspace title bar, confirm that the 4-2H-2V optical configuration is selected.
2. From the menu bar, select **Experiment > New Experiment > Blank Experiment**, then click **OK**.
3. If prompted by the CST Mismatch dialog, select **Use CST Settings**.
4. Rename the experiment with the run date appended with OneFlow (for example, OneFlow Setup_today's date).
5. From the menu bar, select **Experiment > New Specimen**.

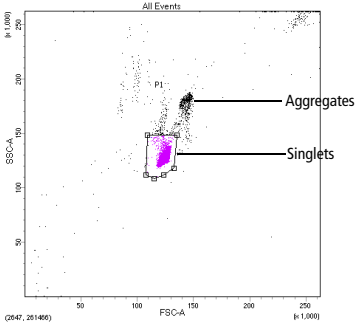
   The **Panel Template** window opens.
6. Click the **BD Panels** tab and select the **OneFlow Setup** template, then click OK.
7. Click **Cytometer Settings** in the Browser window.
8. In the Inspector, select the **Parameters** tab and ensure that **FSC-A**, **FSC-H**, **SSC-A**, and **SSC-H** are all selected.
9. Navigate to the **Compensation** tab in the Inspector and deselect the **Enable Compensation** option.

Adjusting PMTVs

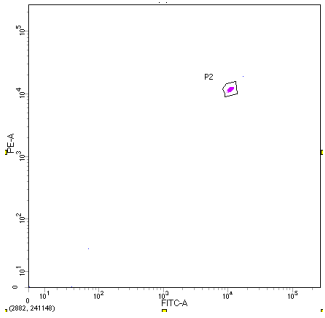
1. In the **Browser**, set the current tube pointer to the BD OneFlow Setup beads tube.
2. In the Acquisition Dashboard, set **Events To Record** to 5,000.
3. Vortex the beads tube.
4. Install the tube on the cytometer.
5. Adjust the flow rate to **Low**, and click **Acquire Data**.

   NOTE It may take 10–15 seconds until events begin to appear.
6. In the FSC-A vs SSC-A dot plot, adjust the **P1** gate to include only the singlet bead population (no aggregates).

   NOTE Click the **Increase** button in the **Tools** menu of the global worksheet to see more detail in the FSC-A vs SSC-A dot plot.



7. In the FITC-A vs PE-A dot plot, adjust the **P2** gate to include only the singlet bead population.



8. In the Cytometer window, select the **Parameters** tab and adjust the voltages for FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-H7, V450, and V500 so that the MFI of the bead population in the P2 gate falls within the corresponding range on the monthly MFI target range card (Figure 1).

Figure 1 Example monthly MFI target range card

BD OneFlow™ Setup Beads (Monthly)

REF LOT

Fluorophore	Min (-2%)	IMFI	Max (+2%)
FITC	10397	10610	10822
PE	11896	12139	12382
PERCP-CY5.5	46584	47535	48486
PE-CY7	22194	22647	23100
APC	57164	58331	59497
APC-H7	129387	132028	134668
V450	9639	9835	10032
V500-C	24076	24568	25059

Monthly Target Ranges

9. If needed, increase the size of the **P2** gate to ensure that the singlet bead population remains within the gate while adjusting the PMTVs.

Experiment Name:	OneFlow Setup_20140627	CYTOMETER CONFIG CRE...	2007-01-02T12:00:00-08:00
Specimen Name:	PMT Setup	CST PERFORMANCE EXPI...	2014-06-24T11:57:03-07:00
Tube Name:	OneFlow Setup Bead_001	CST REGULATORY STAT...	CE-IVD Performance Check
Record Date:	Jun 23, 2014 3:13:44 PM	CST BEADS EXPIRED:	False
CYTOMETER CONFIG NA...	3-laser, 8-color (4-2H-2V) (B...		

Population	FITC-A Median	PE-A Median	PerCP-Cy5-5-A Median	PE-Cy7-A Median
P2	10,654	12,196	47,223	22,513

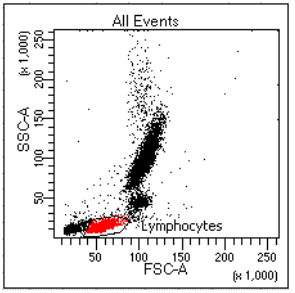
Population	APC-A Median	APC-H7-A Median	V450-A Median	V500-A Median
P2	58,579	132,245	9,751	24,481

10. Click **Record Data**.
11. Verify that the MFI values fall within range.

Adjusting FSC and SSC

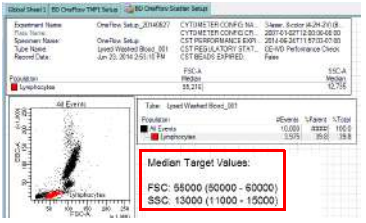
NOTE Use the normal LWB sample that you prepared for this procedure.

1. In the **Browser**, select the current tube pointer for the LWB tube.
2. In the Acquisition Dashboard, confirm that the **Events To Record** are set to 10,000 total events.
3. Vortex the LWB tube.
4. Install the LWB sample on the cytometer and confirm that the flow rate is set to **Low**.
5. Click **Acquire Data**.
6. In the Cytometer window, select the **Parameters** tab and lower the voltages for FSC and SSC so that the lymphocyte population is on scale.
7. In the Cytometer window, select the **Threshold** tab and set the FSC threshold to 10,000.



8. Adjust the Lymphocyte gate to encompass the entire lymphocyte population in the FSC vs SSC dot plot.
9. Adjust the FSC and SSC voltages to place the lymphocyte population within the FSC-A and SSC-A target value ranges given on the BD OneFlow Scatter Setup worksheet. See Figure 2.

Figure 2 Statistics view on worksheet



10. If needed, re-adjust the lymphocyte gate.
11. Click **Record Data**.
12. Verify that the MFI values fall within range.

13. Right-click **Cytometer Settings > Application Settings > Save**, and click **OK**.

    CAUTION Use the default name for the Application Settings. Do not rename the Application Settings.

14. When prompted, click **Yes** to maintain the modified threshold values.

7. LIMITATIONS

- BD OneFlow Setup beads are intended to set voltages appropriate for the BD multicolor tube assay when used with a BD FACSCanto II flow cytometer set with the 4-2H-2V optical configuration and BD FACSDiva software v8.0.1 or later.
- The PMT voltages and Application Settings generated using the BD OneFlow Setup beads are intended to be used for the BD multicolor tube assay and should not be used for any other clinical reagents or assays.
- BD OneFlow Setup beads do not perform as a fluorescence calibrator and should not be used for setting up a flow cytometer for quantitative fluorescence measurements.

8. PERFORMANCE CHARACTERISTICS

Performance of the BD OneFlow Setup beads was established by testing at BD Biosciences laboratories in San Jose, CA.

Accuracy

Accuracy testing was performed using BD FACSDiva software v8.0.1 or later on BD FACSCanto II flow cytometers using BD OneFlow Setup beads (test method), Sphero™* Rainbow calibration particles (reference method), and BD FC beads (used as stable fluorescent particles). On each cytometer, detector gain settings were generated using BD OneFlow Setup beads and Sphero Rainbow calibration particles by placing the beads within the bead lot-specific target MFI ranges specified for each detector. BD FC beads were acquired using each gain setup generated with the test and reference methods. Average MFI of the positive BD FC beads were compared between the test and reference methods. Data is shown in Table 2.

Table 2 Accuracy of MFI values between test and reference methods (relative mean bias)

Channel	% Relative bias	SD[a]
FITC	–0.30	1.16
PE	–0.30	1.43
PerCP-Cy5.5	0.46	2.93
PE-Cy7	1.70	2.25
APC	2.49	3.06
APC-H7	2.25	3.28
V450	–4.41	5.04
V500	0.27	0.85

a. SD= Standard deviation

Precision

Precision testing was performed using BD FACSDiva software v8.0.1 or later on multiple BD FACSCanto II flow cytometers using multiple lots of BD OneFlow Setup beads over multiple days. BD FC beads were used as stable fluorescent particles. Detector gain settings were generated using BD OneFlow Setup beads by placing the beads within the bead lot-specific target

* Sphero is a trademark of Spherotech, Inc.

MFI ranges specified for each detector. Using the PMT gain settings generated for each setup, the eight single color BD FC beads were acquired. Percent CV of the MFI values of the positive BD FC beads were used to verify precision. Data is shown in Table 3.

Table 3 BD OneFlow Setup beads precision (lot to lot and instrument to instrument)

Channel	%CV[a]	UCL[b]
FITC	8.6	10.2
PE	3.4	4.0
PerCP-Cy5.5	17.0	20.2
PE-Cy7	3.9	4.7
APC	1.3	1.5
APC-H7	2.8	3.3
V450	17.1	20.3
V500	4.8	5.7

a. CV = Coefficient of variation
b. UCL = Upper confidence limit of the 95% confidence interval

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.

TROUBLESHOOTING

Problem	Possible Cause	Solution
No beads detected	Beads not mixed prior to diluting	Vortex the beads vial, prepare a fresh suspension of beads according to Table 1, and re-run the tube.
	Beads too dilute	
	Debris in the beads suspension	
	Incorrect beads used	
	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	Clogs within the sample tubes and lines	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Back pressure in the waste lines	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	High scatter noise (FSC or SSC)	Perform monthly maintenance. See the cytometer IFU for more information. Call BD Biosciences.
	FSC threshold is set too high	Lower the FSC threshold.
	FSC and SSC PMTVs are not optimum	Optimize FSC and SSC PMTVs.

Problem	Possible Cause	Solution
No cells detected in lysed, washed blood sample	Air bubbles in the flow cell or sheath filter	Check the fluidics for bubbles and debris. See the cytometer IFU for more information.
	Clogs within the sample tubes and lines	Check the fluidics for clogs and debris. See the cytometer IFU for more information.
	Back pressure in the waste lines	Check the waste tank vent for obstructions. See the cytometer IFU for more information.
	Cell concentration in prepared samples is too low	Prepare a new sample.
	FSC and SSC PMTVs not optimum for cells	Optimize FSC and SSC PMTVs.

REFERENCES

1. *Protection of Laboratory Workers from Occupationally Acquired Infections—Third Edition; Approved Guideline*. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document M29 A-3.
2. Centers for Disease Control. Perspectives in disease prevention and health promotion 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.

BD OneFlow™ LST

20 Tests per kit—Catalog No. 658619

CE IVD

23-15586-03
1/2021

CONTENTS

1. INTENDED USE

The BD OneFlow™ LST (Lymphoid Screening Tube) is intended for flow-cytometric immunophenotyping of normal and aberrant mature lymphocyte populations of B, T, and NK lineages in peripheral blood, bone marrow, and lymph nodes, as an aid in the diagnosis of hematological disorders. The BD OneFlow™ LST is designed for use with a suitably equipped BD flow cytometer and software designated for in vitro diagnostic use.

2. SUMMARY AND EXPLANATION

In chronic lymphoproliferative disorders (CLPD), clonogenic events lead to the expansion and accumulation of mature-appearing lymphocytes, which carry a proliferative and/or survival advantage over their normal counterparts.[1] Thus, the detection of phenotypically aberrant and clonal mature lymphocytes is critical to the diagnosis of CLPD.

The EuroFlow™* Consortium designed multicolor antibody panels to fully characterize the cell populations in a patient specimen using immunophenotypic markers that are indicative of normal and abnormal cells.[1] In addition to the optimized multicolor antibody panels, the EuroFlow protocol comprises standardized procedures for cytometer setup, determination of assay settings, sample preparation and staining, sample acquisition, and data analysis.[2]

The single-tube screening panels and multi-tube classification panels fit into the EuroFlow diagnostic algorithm for the identification and classification of hematological disorders. Each tube contains a set of backbone markers and a set of classification markers.[1] Backbone markers are shared across a particular set of panels and are used to normalize the samples so that data files can be combined and analyzed

* The EuroFlow trademark and logo and the EuroFlow™ antibody panels are property of the EuroFlow Consortium and cannot be reproduced or published without prior written permission from the EuroFlow coordinator (www.euroflow.org).

as a single large data file. They are markers that identify distinct cell populations in a particular cell lineage. Classification markers have been selected for their diagnostic utility in discriminating between cell types within a given lineage and in classifying the abnormal cell type in the sample.

3. PRINCIPLES OF THE PROCEDURE

Multiparameter flow cytometry is a sensitive and rapid tool for the qualitative and quantitative characterization of cell populations in a specimen. Cells are incubated with fluorochrome-conjugated antibodies which bind to their target molecules. The stained cells can then be analyzed on a single-cell basis. Multiparameter analysis of the data is used to identify the cell populations in the patient specimen and can lead to the identification of an aberrant clonal cell population.

The number of parameters used in flow cytometric immunophenotyping of hematological disorders has increased in recent years. BD OneFlow™ LST contains a panel of fluorochrome-conjugated antibodies that identify normal and aberrant populations of B, T, and NK lymphocytes. Analysis of the dot plots allows for the identification of normal and abnormal cell populations.

4. REAGENT

Reagent Composition

BD OneFlow™ LST consists of single-use tubes containing the following fluorochrome-conjugated antibodies in an optimized dried formulation. See Table 1.

Table 1 BD OneFlow™ LST antibody panel

Antibody	Fluorochrome	Clone	Isotype
CD8	FITC	SK1 (Leu2a)[3]	IgG_1, κ

Table 1 BD OneFlow™ LST antibody panel

Antibody	Fluorochrome	Clone	Isotype
Anti-Lambda	FITC	1-155-2[4]	IgG_1, κ
CD56	PE	MY31 (Leu-19)[5,6]	IgG_1, κ
Anti-Kappa	PE	TB28-2[4]	IgG_1, κ
CD5	PerCP-Cy™5.5[a]	L17F12[7,8]	IgG_{2a}, κ
CD19	PE-Cy™7	SJ25-C1[9,10]	IgG_1, κ
Anti–TCRγ/δ-1	PE-Cy7	11F2[11,12]	IgG_1, κ
CD3	APC	SK7[8]	IgG_1, κ
CD38	APC-H7	HB7[9]	IgG_1, κ
CD4	V450[b]	SK3 (Leu3a)[3]	IgG_1, κ
CD20	V450	L27[9]	IgG_1, κ
CD45	V500-C[b]	2D1[13,14]	IgG_1, κ

a. Cy™ is a trademark of GE Healthcare. This product is subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and is made and sold under license from GE Healthcare. This product is licensed for sale only for in vitro diagnostics. It is not licensed for any other use. If you require any additional license to use this product and do not have one, return this material, unopened, to BD Biosciences, 2350 Qume Drive, San Jose, CA 95131, and any money paid for the material will be refunded.

b. BD Horizon™ V450, BD Horizon™ V500-C

The antibodies in BD OneFlow™ LST were chosen for their ability to separate normal lymphocytes into their major subpopulations.

CD45 identifies mature lymphocytes and B-cell precursors.

CD3 identifies T cells. CD3 can also be used to identify B cells and NK cells by exclusion.

Anti-TCRγ/δ-1, CD5, CD4, and CD8 can separate T cells into a number of subpopulations.

CD19 and CD20 identify B cells, and together with CD45 can separate B cells into mature B lymphocytes ($CD19^+$, $CD20^{hi}$, $CD45^{hi}$) and B-cell precursors ($CD19^+$, $CD20^{-/lo}$, $CD45^{lo}$). CD19 and CD20 are also used to identify NK cells by exclusion.

Anti-Kappa and Anti-Lambda can identify normal and clonally expanded populations of B cells expressing Igκ or Igλ on the surface membrane, respectively.

CD38 identifies plasma cells and B-cell precursors. In addition, it is informative in the evaluation of a wide variety of lymphoid malignancies. CD38 can also aid in the identification of NK cells.

CD56 identifies NK cells.

Refer to the article describing the EuroFlow antibody panels[1] for a full description of the utility of the antibodies chosen for BD OneFlow™ LST.

Precautions

- The reagent contains 0.25 – <1% of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (3:1) (CAS number 2682-20-4) and 0.1 – <0.25% of sodium azide (CAS number 26628-22-8). The reagent is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Go to regdocs.bd.com to download the Safety Data Sheet.

	Warning
	H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects.

	Warning
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection. P273: Avoid release to the environment.
Response	P302+P352: IF ON SKIN: Wash with plenty of water/... P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P321: Specific treatment (see Safety Data Sheet). P363: Wash contaminated clothing before reuse.
Disposal	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.

Storage and Handling

Store tubes at 2°C–27°C in the foil pouch. Do not freeze the reagent or expose it to direct light at any time during storage or incubation with cells. The dried fluorochrome-conjugated antibodies are stable until the expiration date shown on the pouch and tube labels when stored as directed. Do not use after the expiration date. Once the pouch is opened, the dried fluorochrome-conjugated antibodies are stable for one month when stored as directed.

CAUTION Ensure the pouch is completely resealed after removing a tube. The reagent is very sensitive to moisture. Do not remove the desiccant from the reagent pouch.

5. INSTRUMENTS

BD OneFlow™ LST is for use on the following BD instruments.

Table 2 Recommended BD instruments

Flow cytometer	Setup beads	Setup software	Analysis software
BD FACSLyric™[a]	BD® CS&T Beads BD® FC Beads 7-Color Kit BD® FC Beads 5-Color Kit	BD FACSuite™ Clinical application v1.4 or later	BD FACSuite™ Clinical application v1.4 or later
BD FACSCanto™ II[b]	BD FACSDiva™ CS&T IVD Beads BD OneFlow™ Setup Beads BD® FC Beads 8-Color Kit for BD OneFlow™ Assays	BD FACSDiva™ software v8.0.1 or later	BD FACSDiva™ software v8.0.1 or later

a. 8-color (4-Blue 2-Red 2-Violet), 10-color (4-Blue 3-Red 3-Violet), or 12-color (4-Blue 3-Red 5-Violet)
b. 3-laser, 8-color, 4-2H-2V BD default (4-2H-2V) optical configuration

The BD FACS™ Universal Loader can be used with this product. See the *BD FACSLyric™ Clinical System Instructions For Use* for more information. The Loader can be used with BD FACSuite™ Clinical application v1.5 or later.

6. SPECIMENS

BD OneFlow™ LST can be used for immunophenotyping by flow cytometry of peripheral blood (PB) or bone marrow (BM) aspirates collected in EDTA or heparin[15-18,21-23] (for example, BD Vacutainer® blood collection tubes), and fresh lymph nodes (LN) collected in PBS or cell culture media, such as RPMI. Each type of specimen can have different storage conditions and limitations that should be considered prior to collection and analysis.[15,16,21]

Specimens should be processed immediately after collection, or up to 24 hours after collection if stored at room temperature (20°C–25°C).[17,18,22,23] If a longer period of time is needed, each laboratory should validate that specimens processed and stored according to their procedures produce equivalent results to specimens processed immediately after collection.

Specimens with large numbers of nonviable cells can give erroneous results due to selective loss of populations and to increased nonspecific binding of antibodies to nonviable cells. Viability of specimens should be assessed and a cutoff value established. A cutoff value of at least 80% viable cells has been suggested.[15]

Samples should be acquired within 60 minutes if kept at room temperature, protected from light. If a longer period of time is needed, each laboratory should validate that stained specimens acquired after being held under their storage conditions produce equivalent results to specimens acquired immediately after staining. Protect stained specimens from light until they are acquired.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[19,20] 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.

7. REAGENTS AND MATERIALS

Reagents Provided

BD OneFlow™ LST is provided as single-use tubes in foil pouches. Each kit contains four pouches, each containing five tubes of BD OneFlow™ LST.

Reagents and Materials Required but Not Provided

For BD FACSLyric™ flow cytometers:

- BD OneFlow™ Assays Installer I (Catalog No. 664225)

  An installer is required for the BD OneFlow™ LST assay. If you are using the Loader, use BD OneFlow™ Assays Installer I v1.1 to install the assay in BD FACSuite™ Clinical application v1.5 or later. The assay comprises an acquisition sheet, a laboratory report, a physician report, and a supplemental report used for further investigation. Unless you already have the current OneFlow LST assay, you will have to order the installer the first time you order BD OneFlow™ LST. The installer also contains assays for other BD OneFlow™ reagents.

  The *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers* is provided with the installer. Application guides for other BD OneFlow™ reagents are also included.

For BD FACSCanto™ II flow cytometers:

- BD OneFlow™ Assay Templates Installer (Catalog No. 659305)

  An installer is required for the BD OneFlow™ LST template. The template contains two global worksheets: the BD OneFlow™ LST Acquisition worksheet and the BD OneFlow™ LST Analysis worksheet. Unless you already have the current BD OneFlow™ LST template, you will have to order the installer the first time you order BD OneFlow™ LST. The installer also contains the OneFlow Setup template and templates for other BD OneFlow™ reagents.

  The *Instrument Setup Guide for BD OneFlow™ Assays* and the *BD OneFlow™ LST Application Guide* are provided with the installer. Application guides for other BD OneFlow™ reagents are also included.

- 15-mL conical polypropylene tubes

- Pasteur pipet
- Serological pipet
- Micropipettor with tips
- Vortex mixer
- Centrifuge
- Wash buffer (filtered PBS + 0.5% BSA + 0.09% or 0.1% sodium azide)
- BD FACS™ Lysing Solution (10X) (Catalog No. 349202)

  See the *BD FACS™ Lysing Solution* instructions for use (IFU) for precautions and warnings.

For BD FACSLyric™ flow cytometers:

- BD® CS&T Beads (Catalog No. 656504 or 656505)
- BD® FC Beads 7-Color Kit (Catalog No. 656867)
- BD® FC Beads 5-Color Kit (Catalog No. 661564)

For BD FACSCanto™ II flow cytometers:

- BD FACSDiva™ CS&T IVD Beads (Catalog No. 656046 or 656047)
- BD OneFlow™ Setup Beads (Catalog No. 658620)
- BD® FC Beads 8-Color Kit for BD OneFlow™ Assays (Catalog No. 658621)

8. PROCEDURE

Installing the Assay or Template

The BD OneFlow™ LST assay, used with BD FACSuite™ Clinical application, or the BD OneFlow™ LST template, used with BD FACSDiva™ software, has to be installed before you run the assay for the first time. Additional assays or templates can be installed at the same time, as needed. If you will analyze the FCS files on a different workstation from the one used to acquire the samples, ensure that you install the assays or templates on both workstations.

To install the BD OneFlow™ assay in BD FACSuite™ Clinical application:

NOTE When you select an assay to install, it will overwrite the BD OneFlow™ LST assay that was previously installed on the system. If you do not want an existing assay on your computer to be overwritten, do not select that assay from the installer during the installation process.

1. Insert the installer and click the installer icon.

   The InstallShield Wizard for BD OneFlow™ Assays opens.

2. Click **Next**.

   The license agreement opens.

3. Select the **I accept the terms in the license agreement** option and click **Next**.

4. To install all of the assays included on the installer, select the **Complete** option and click **Next**.

5. Optional: To install a subset of the assays included on the installer, select the **Custom** option and click **Next**.

   The **Custom Setup** dialog opens.

   - Click the menu to the left of the appropriate assay.
   - From the menu, select **This assay will be installed on your local hard drive**.

6. Click **Install**.

   The assays will be installed in the Library.

7. Click **Finish**.

   The InstallShield Wizard closes.

8. Optional: Double-click the ReadMe file found on the installer.

   The ReadMe file opens.

9. Click the close box when finished reading it.

10. Remove the installer.

To install the OneFlow template in BD FACSDiva™ software:

NOTE When you select a template to install, it will always overwrite any template with the same name that was previously installed on the system. If you do not want an existing template on your computer to be overwritten, do not select that template from the installer during the installation process.

1. Insert the installer and click the installer icon.

2. Follow the instructions in the dialog.

   The installer will copy and paste the templates in the folder D:\BDExport\Templates\Panel\BD Panels.

   NOTE If your system has only one drive, the templates will be installed in C:\BDExport\Templates\Panel\BD Panels.

   After installation is complete, a dialog opens, summarizing which templates have been successfully copied into the folder.

3. Click **OK** to close the dialog.

4. The installer ReadMe file opens. Click the close box when you have finished reading it.

5. Remove the installer.

Setting up the Cytometer

For BD FACSLyric™ flow cytometers:

1. Use BD® CS&T Beads and BD FACSuite™ Clinical application v1.4 or later, to perform Characterization QC (CQC) every 6 months or as needed, perform daily Performance QC (PQC), and

perform daily assay and tube settings setup. For assay and tube settings setup, select the **Run Setup** and **Generate Reports** checkboxes.

2. Use the BD® FC Beads 7-Color Kit, BD® FC Beads 5-Color Kit, and BD FACSuite™ Clinical application v1.4 or later, to update reference settings every 60 days.

   See the *BD FACSLyric™ Clinical System Instructions For Use*, the *BD FACSLyric™ Clinical Reference System*, and the appropriate reagent IFU for more information.

For BD FACSCanto™ II flow cytometers:

1. Use BD FACSDiva™ CS&T IVD Beads (CS&T IVD beads) and BD FACSDiva™ software v8.0.1 or later, to define the baseline of the cytometer and to run a daily performance check of the cytometer.
2. Use BD OneFlow™ Setup Beads, lysed washed blood, and BD FACSDiva™ software v8.0.1 or later, to set photomultiplier tube (PMT) and scatter voltages monthly.
3. Use BD® FC Beads and BD FACSDiva™ software v8.0.1 or later, to set fluorescence compensation monthly.
4. We recommend that you confirm that the PMT voltages (PMTVs) are still within their daily target ranges.

   See the *Instrument Setup Guide for BD OneFlow™ Assays* and the appropriate reagent IFU for more information.

Diluting BD FACS™ Lysing Solution

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.

Processing the Specimen

Washing the specimen

NOTE Before washing the specimen, confirm that the cytometer has been properly set up.

1. Label a 15-mL conical tube with the specimen ID.
2. Invert the specimen in the collection tube 10 times to mix well.
3. Add 300 µL of the specimen to the labeled conical tube.
4. Add 10 mL of wash buffer (filtered PBS + 0.5% BSA + 0.09% or 0.1% sodium azide).
5. Invert the tube 3–5 times to mix well.
6. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
7. Remove the supernatant without disturbing the cell pellet.
8. Vortex the tube until no cell aggregates remain before adding wash buffer.
9. Repeat steps 4–8 twice for a total of three washes.
10. Resuspend the cell pellet in 200 µL of wash buffer to give a final volume of approximately 300 µL.

    NOTE Start staining the specimen using BD OneFlow™ LST within 30 minutes of the last wash. Store the washed specimen at 20°C–25°C until you stain it.

Staining the specimen

1. If the pouch is stored refrigerated, allow it to reach room temperature before opening it.

    NOTE The reagent is very sensitive to moisture. To avoid condensation, open the pouch only if it is at room temperature.

2. For each patient specimen, remove a BD OneFlow™ LST tube from the pouch.
3. Place the tubes in a rack, protected from light.

   Start staining the specimen within one hour of removing a tube from the pouch.
4. Immediately reseal the pouch with any unused tubes.

   NOTE Ensure the pouch is completely resealed after removing a tube. The reagent is very sensitive to moisture. Do not remove the desiccant from the reagent pouch.
5. Write the patient ID on the BD OneFlow™ LST tube label within the area provided.

   NOTE Write the current date on the pouch label when it is first opened. Use the tubes from that pouch within one month before opening the next pouch.
6. Vortex washed specimen 3–5 seconds to mix well.
7. Add 100 µL of washed specimen to the tube. Vortex vigorously 3–5 seconds to mix well.

   If less than 100 µL of specimen is used, add wash buffer to a final volume of 100 µL.

   NOTE Do not wipe the outside of the tube with ethanol or isopropanol because the ink on the printed label can run.
8. Incubate for 30 minutes at 20°C–25°C, protected from light.
9. Add 2 mL of 1X BD FACS™ Lysing Solution. Vortex 3–5 seconds to mix well.
10. Incubate for 10 minutes at 20°C–25°C, protected from light.
11. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.

12. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
13. Vortex vigorously until the cell pellet is completely resuspended.
14. Add 2 mL of wash buffer to the tube. Vortex 3–5 seconds to mix well.
15. Centrifuge at 540*g* for 5 minutes at 20°C–25°C.
16. Remove the supernatant without disturbing the cell pellet, leaving approximately 50 µL of residual liquid in the tube.
17. Vortex 3–5 seconds to resuspend the cell pellet.
18. Add 200 µL of wash buffer to the tube. Vortex 3–5 seconds to mix well.

    NOTE Samples should be acquired within 60 minutes if kept at room temperature, protected from light. If a longer period of time is needed, each laboratory should validate that stained specimens acquired after being held under their storage conditions produce equivalent results to specimens acquired immediately after staining. Protect stained specimens from light until they are acquired.

Setting up the Assay (BD FACSLyric™ Flow Cytometer)

To add a reagent lot ID and expiration date to the library:

1. From the BD FACSuite™ Clinical application navigation bar, click the Library icon.

   The Library workspace opens.
2. Expand the **Beads and Reagents** menu and select **Reagents**.
3. Select OneFlow LST from the **Product Name** list.

   The **OneFlow LST** pane opens at the bottom of the page.

4. Click **Add Lot**.

   The **Add New Lot** dialog opens.

5. In BD FACSuite™ Clinical application v1.5, click **Scan Barcode** and then scan the barcode on the pouch or tube label.

   The Lot ID and expiration date are entered in the appropriate fields.

   NOTE In BD FACSuite™ Clinical application v1.4, add the Lot ID and expiration date manually.

6. Select the **Current Lot** checkbox.

7. Click **OK**.

   The lot ID and expiration date are added to the appropriate columns for the reagent.

   NOTE Make sure to add the reagent lot and expiration date prior to acquisition. This has to be done only once for a particular reagent lot.

To create a worklist:

1. From the BD FACSuite™ Clinical application navigation bar, click the Worklists icon.

   The Worklists workspace opens.

2. In the **Manage Worklists** tab, click **New**.

   A blank worklist opens in a new tab.

3. In the **Worklist Entries** section, select the appropriate task from the **Task** menu.

4. Enter the **Sample ID** for BD OneFlow™ reagent tasks.

   Do not scan the barcode, found on the tube label, into the software.

   NOTE Multiple lots of the same reagent cannot be run on the same worklist.

5. In the **Loading Options** section, select **Manual** or **Universal Loader** from the **Loading Option** menu.

6. If using the Loader, select **30 Tube Rack** or **40 Tube Rack** from the **Carrier Type** menu.

   See the *BD FACSLyric™ Clinical System Instructions For Use* for more information.

Setting up the Experiment (BD FACSCanto™ II Flow Cytometer)

1. From the menu bar, select **Edit > User Preferences,** then navigate to the **FCS** tab, and select **Export FCS after recording,** to automatically export the FCS files after acquisition. Click **OK.**

2. Confirm that the cytometer is in the default 4-2H-2V configuration.

3. From the menu bar, select **Experiment > New Experiment > Blank Experiment**. Click **OK**.

   NOTE You can also create an experiment directly from the **Browser** using the **Experiment** icon.

4. If prompted by the **CST Mismatch** window, select **Use CST Settings**.

5. Rename the experiment according to your laboratory practice.

6. In the **Browser**, right-click **Cytometer Settings > Link Setup** and select the appropriate compensation matrix calculated using BD® FC Beads within the past 31 days. Click **Link**.

   See the *BD® FC Beads 8-Color Kit for BD OneFlow™ Assays* IFU or the *Instrument Setup Guide for BD OneFlow™ Assays*.

7. If prompted by the **Cytometer Settings Mismatch** window, select **Overwrite**.
8. Right-click **Cytometer Settings > Unlink From** the previously linked compensation setup. Click **OK**.

   NOTE Unlinking the compensation setup allows updated application settings to be applied while retaining compensation values.

9. In the **Browser**, right-click **Cytometer Settings > Application Settings > Apply** and select the most recent application settings determined within the last 31 days using the BD OneFlow™ Setup Beads. Click **Apply**.
10. A **Confirm** dialog opens. Select **Keep the compensation value**.
11. If prompted by the **Confirm Cytometer Changes** dialog, click **Yes** to overwrite the cytometer values for **FSC Area Scaling**.
12. From the menu bar, select **Experiment > New Specimen**.

    The **Panel Templates** dialog opens.

13. Navigate to the **BD Panels** tab and select the OneFlow LST template.
14. Indicate the number of patient specimens you want to acquire using the **Copies** field near the bottom of the **BD Panels** tab. Click **OK**.

15. Rename each specimen, for example, with the appropriate patient ID in front of the specimen name.

    NOTE If you have to re-run a particular patient sample, set the current tube pointer to the tube you wish to re-run. Click **Next Tube** in the **Acquisition Dashboard** to create another tube for that patient. Do not select **Experiment > New Tube** from the menu bar or use the **New Tube** icon from the **Browser** menu bar to create the additional tube to be acquired because the labels and barcode fields will not be populated.

    NOTE If you want to acquire additional patient samples stained with BD OneFlow™ LST in the experiment, repeat steps 12–15 to add new specimens. Two **Confirm** dialogs will open asking if you want to create another LST acquisition worksheet or another LST analysis worksheet. Click **Cancel** in each dialog.

16. From the menu bar, select **Experiment > Experiment Layout** and navigate to the **Keywords** tab.

17. Highlight the **Product ID** keyword for the appropriate tube, and scan the barcode on the BD OneFlow™ LST tube label.

    NOTE If you cannot scan the barcode on the tube label, see Troubleshooting.

18. Manually add the appropriate information to the remaining keywords, as needed.

19. Click **OK** to close the **Experiment Layout**.

Acquiring the Stained Sample

For BD FACSLyric™ flow cytometers:

Two versions of the assay are available:

Assay version	Software	Stopping time	Acquisition mode
v1.0	BD FACSuite™ Clinical application v1.4 or later	5 minutes	Manual
v1.1	BD FACSuite™ Clinical application v1.5 or later	3 minutes	Manual, Loader

The assay will automatically collect 100,000 total events. You cannot append the number of events to collect after acquisition has started. Therefore, if needed, change the number of events to collect before you start acquisition. To change the number of events to collect, see the *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers*. A clinically relevant number of cells can be determined at the discretion of an appropriate healthcare professional.

To acquire the sample using BD OneFlow™ LST assay v1.1:

1. In the **Worklist Controls** bar, select **Run All** from the **Run** menu to run the entire worklist from the beginning.

   Alternatively, to acquire a specific tube, set the run pointer to the sample you want to run and select **Run from Pointer** from the **Run** menu.

2. Vortex each stained tube 3–5 seconds at low speed immediately prior to acquisition.

   If using the BD FACS™ Universal Loader, vortex tubes immediately before placing them in the Loader racks.

   NOTE Make sure that all of the BD OneFlow™ tubes in the rack are acquired within 1 hour. If not, you must validate tubes acquired outside the 1 hour time.

3. Follow the prompts in the software to load or unload tubes.

   The BD OneFlow™ LST Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Leukocytes, Lymphocytes, B cells, T cells, NK cells, and their relevant subpopulations.

4. Examine each dot plot on the acquisition sheet.

   NOTE The preview time is 10 seconds and then data is automatically recorded. Do not increase the preview time and risk loss of the sample due to insufficient volume.

   NOTE The assay will automatically collect 100,000 total events. If the assay cannot collect 100,000 total events, acquisition will stop after 3 minutes. A QC message, "All Events gate does not contain the requested 100,000 events" is generated in the Lab Report, and can be ignored if the sample can be analyzed using the events acquired.

   See the *BD FACSLyric™ Clinical System Instructions For Use* for more information.

To acquire the sample using BD OneFlow™ LST assay v1.0:

1. In the **Worklist Controls** bar, select **Run All** from the **Run** menu to run the entire worklist from the beginning.

   Alternatively, to acquire a specific tube, set the run pointer to the sample you want to run and select **Run from Pointer** from the **Run** menu.

2. Vortex each stained tube 3–5 seconds at low speed immediately prior to acquisition.

3. Follow the prompts in the software to load or unload tubes.

   The BD OneFlow™ LST Acquisition sheet opens. The acquisition sheet contains dot plots and gates to identify Leukocytes,

Lymphocytes, B cells, T cells, NK cells, and their relevant subpopulations.

4. Examine each dot plot on the acquisition sheet.

   NOTE The preview time is 10 seconds and then data is automatically recorded. Do not increase the preview time and risk loss of the sample due to insufficient volume.

5. If it appears that fewer than 100,000 events will be collected, monitor the sample volume and click **Stop Tube** in the **Worklist Controls** bar to stop acquisition before the tube runs dry.

   NOTE The assay will automatically collect 100,000 total events. If the assay cannot collect 100,000 total events, acquisition will stop after 5 minutes. However, make sure you monitor the sample volume and click **Stop Tube** in the **Worklist Controls** bar to stop acquisition before the tube runs dry. To change the stopping criteria, see the *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers*.

   See the *BD FACSLyric™ Clinical System Instructions For Use* for more information.

For BD FACSCanto™ II flow cytometers:

1. In the **Browser**, expand the appropriate specimen and set the current tube pointer to that tube.
2. Select the **BD OneFlow™ LST Acquisition** worksheet tab.
3. Vortex the stained tube 3–5 seconds at low speed.
4. Install the tube on the cytometer. Adjust the flow rate to **Medium** in the **Acquisition Dashboard**. Click **Acquire Data**.
5. Verify that the population is on scale and adjust the gate in the first plot of the acquisition worksheet to exclude debris, if needed.

6. Click **Record Data** in the **Acquisition Dashboard** to collect total events.

   NOTE The template will automatically collect 100,000 total events. Use the menu in the **Acquisition Dashboard** to select a different number of events to acquire, if needed. A clinically relevant number of cells can be determined at the discretion of an appropriate healthcare professional.

7. Inspect the dot plots on the acquisition worksheet and adjust the gates as needed.

   Some of the dot plots might look different from those in other experiments. The initial FSC-A vs SSC-A dot plot to identify cells and eliminate debris may appear compressed. This is a consequence of the target values used to create the application settings. The values are specified by the EuroFlow Consortium.

   NOTE Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size.

   The FSC-A vs SSC-A dot plot is used to identify cells.

   The CD45 V500-A vs SSC-A dot plot contains two gates to identify leukocytes and lymphocytes. T cells and B cells are identified in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the lymphocyte population.

   T cells are divided into TCRγ/δ^+ and TCRγ/δ^- populations in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the T-cell population. TCRγ/δ^- cells are divided into $CD8^+CD4^-$ and

CD4+CD8– populations in the CD20+CD4 V450-A vs CD8+IgL FITC-A dot plot.

Igκ- and Igλ-expressing B cells are identified in the CD56+IgK PE-A vs CD8+IgL FITC-A dot plot from the B-cell population.

NK cells are identified from the NOT(T cells OR B cells) population in the CD45 V500-A vs CD56+IgK PE-A dot plot.

The remaining dot plots do not contain gates and are included to ensure that the antibodies can stain cells in the specimen, therefore serving as an internal quality control for the tube.

NOTE See the *BD OneFlow™ LST Application Guide* for examples of the dot plots showing populations of normal cells in the LST acquisition worksheet.

8. Acquire the next sample.
9. From the menu bar, select **File > Export > Experiments**, and select the **Directory Export** option. Click **OK**.

Analyzing the Data Using BD FACSuite™ Clinical Application

NOTE FCS files acquired using BD OneFlow™ LST assay v1.0 can be opened in BD OneFlow™ LST assay v1.1.

1. Set the run pointer to the appropriate sample in the **Worklist Entries** panel.

   The BD OneFlow™ LST Laboratory Report opens in the **Laboratory Report** tab.

2. Review the BD OneFlow™ LST Laboratory Report.

   The first page of the laboratory report shows sample and tube information, population statistics, and QC messages, if generated.

   NOTE Populations with a low number of events might report %Parent or %Grandparent as 0.0%. This is due to rounding the

result to a single decimal place in BD FACSuite™ Clinical application.

3. Inspect the dot plots on page 2 of the laboratory report and adjust the gates as needed.

   The gates in the dot plots of the laboratory report are provided for analyzing normal and aberrant cell populations in the specimen.

   NOTE Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size.

   The dot plots on page 2 of the report provide a high level cell analysis.

   The first three dot plots on the report identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

   Leukocytes and lymphocytes are identified in the CD45 V500-A vs SSC-A dot plot from the SSC Singlets population.

   B cells ($CD3^{-}CD19^{+}$) and T cells ($CD3^{+}$) are identified in the CD3 APC-A vs CD19/TCRgd PE-Cy7-A dot plot from the Lymphocytes population.

   $TCR\gamma\delta^{+}$ T cells and $TCR\gamma\delta^{-}$ T cells are identified in the CD3 APC-A vs CD19/TCRgd PE-Cy7-A dot plot from the T cells population.

   See the *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers* for examples of dot plots showing populations of normal cells.

4. Inspect the dot plots on page 3 of the laboratory report and adjust the gates as needed.

   The dot plots on page 3 of the laboratory report are used to analyze B cells. Examine the level of CD20 expression in the CD19/TCRgd PE-Cy7-A vs CD20/CD4 V450-A dot plot.

   Examine the ratio of Igκ- to Igλ-expressing B cells in the CD56/IgK PE-A vs CD8/IgL FITC-A dot plot.

   The remaining dot plots further characterize B cells using various markers.

5. Inspect the dot plots on page 4 of the laboratory report and adjust the gates as needed.

   The dot plots on page 4 of the laboratory report identify various populations of T cells. TCRγ/δ$^-$ T cells are divided into CD4$^+$CD8$^-$, CD8$^+$CD4$^-$, CD4$^+$CD8$^+$, and CD4$^-$CD8$^-$ populations in the CD20/CD4 V450-A vs CD8/IgL FITC-A dot plot.

   The CD4$^+$ and CD8$^+$ populations of T cells might trail into the double positive quadrant instead of being discrete populations. This is a consequence of the panel of antibodies present in BD OneFlow™ LST.

   The remaining dot plots further characterize TCRγ/δ$^-$ and TCRγ/δ$^+$ cells using various markers.

6. Inspect the dot plots on page 5 of the laboratory report and adjust the gates as needed.

   The dot plots on page 5 of the laboratory report identify NK cells. NK cells are identified from the NOT T cells OR B cells population in the CD45 V500-C-A vs CD56/IgK PE-A dot plot.

   The remaining dot plots further characterize NK cells using various markers.

7. Inspect page 6 of the laboratory report.

   Page 6 of the laboratory report includes lot and expiration dates for BD® CS&T Beads and the BD OneFlow™ reagent, reference settings, tube settings, and cytometer configuration.

8. (Optional) Select the **Physician Report** tab to view the report.

   The OneFlow LST Physician Report contains a high level summary of the assay results.

9. (Optional) Select the **Supplemental Report** tab to add additional dot plots to further analyze the sample.

   See the *BD OneFlow™ LST Application Guide for BD FACSLyric™ Flow Cytometers* for more information.

   WARNING Any gated regions deleted in this Supplemental Report are reflected in the Laboratory and Physician Reports. Any gated regions created in this Supplemental Report might be reflected in the Laboratory Report.

   WARNING Do not add dot plots or gates to the Laboratory Report or Physician Report. They cannot be deleted and will invalidate the report.

10. Select the **Laboratory Report** tab.

11. Click **E-sign**.

    The **E-Signature** dialog opens.

12. Select a user ID.

13. Type your password.

14. (Optional) Enter any comments.

15. Click **Sign**.

    The dialog closes and the signer's user ID, date and time, and comments are added to the E-signature box in all three reports.

16. Click **Approve**.

    The Laboratory and Physicians Reports are automatically exported to C:\BD Export Clinical. If needed, manually export the Supplemental Report.

    See the *BD FACSLyric™ Clinical System Instructions For Use* for more information and export options.

Analyzing the Data Using BD FACSDiva™ Software

1. From the menu bar, select **File > Import > Experiments**.
2. Select the experiment that you want to analyze. Click **Import**.

   The experiment with the associated acquisition and analysis worksheets opens.
3. Select the **BD OneFlow™ LST Analysis** worksheet tab.
4. Inspect the dot plots on page 1 of the LST analysis worksheet and adjust the gates as needed.

   Some of the dot plots might look different from those in other experiments. The initial FSC-A vs SSC-A dot plot to identify cells and eliminate debris may appear compressed. This is a

consequence of the target values used to create the application settings. The values are specified by the EuroFlow Consortium.

NOTE Enlarge the dot plots while adjusting the gates so you can more readily see the populations of interest. After adjusting the gates, collapse the dot plot to its original size.

The first three dot plots on the LST analysis worksheet identify cells, FSC singlets, and SSC singlets. Debris and doublets are excluded by adjusting the gates.

Examine the leukocyte and lymphocyte populations in the CD45 V500-A vs SSC-A dot plot.

Examine the B-cell and T-cell populations in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the lymphocyte population. Examine the TCRγ/δ$^+$ and TCRγ/δ$^-$ populations in the CD3 APC-A vs CD19+TCRgd PE-Cy7-A dot plot from the T-cell population. The CD38 APC-H7-A vs SSC-A dot plot is included for informational purposes to allow for the visualization of CD38$^+$ cells.

NOTE See the *BD OneFlow™ LST Application Guide* for examples of dot plots showing populations of normal cells.

5. Inspect the dot plots on page 2 of the LST analysis worksheet and adjust the gates as needed.

   The dot plots on page 2 of the analysis worksheet identify various populations of T cells. TCRγ/δ$^-$ T cells are divided into CD8$^+$CD4$^-$, CD4$^+$CD8$^+$, CD4$^+$CD8$^-$, and CD4$^-$CD8$^-$ populations in the CD20+CD4 V450-A vs CD8+IgL FITC-A dot plot.

   The remaining dot plots further characterize TCRγ/δ$^-$ and TCRγ/δ$^+$ cells using various markers.

6. Inspect the dot plots on page 3 of the LST analysis worksheet and adjust the gates as needed.

   The dot plots on page 3 of the analysis worksheet identify B cells. B cells are initially identified as being $CD3^{-}CD19^{+}CD45^{+}$. Examine the level of CD20 expression in the CD19+TCRgd PE-Cy7-A vs CD20+CD4 V450-A dot plot.

   Examine the ratio of Igκ- to Igλ-expressing B cells in the CD56+IgK PE-A vs CD8+IgL FITC-A dot plot.

   The remaining dot plots further characterize B cells using various markers.

7. Inspect the dot plots on page 4 of the LST analysis worksheet and adjust the gates as needed.

   The dot plots on page 4 of the analysis worksheet identify NK cells. NK cells are identified from the NOT(T cells OR B cells) population in the CD45 V500-A vs CD56+IgK PE-A dot plot.

   The remaining dot plots further characterize NK cells using various markers.

8. Examine the results in the statistics box on page 5 of the LST analysis worksheet.

   Confirm that all of the keywords are present in the statistics box. If any of the keywords are missing, see Troubleshooting.

9. Perform further analyses as needed.

   NOTE The gates in the dot plots of the LST analysis worksheet are provided for analyzing normal and aberrant cell populations in the specimen.

10. Save the LST analysis worksheet as a PDF.

    NOTE The analysis worksheet is a global worksheet. Any gates that are adjusted when analyzing a sample on a global worksheet

will be changed in previously analyzed files. Previously saved PDFs won't change, but if you go back to a previously analyzed global worksheet, you will have to readjust the gates so they match what they were before.

11. (Optional) Click **Print** to print the LST analysis worksheet.
12. Analyze the next sample.

9. LIMITATIONS

- Use of therapeutic monoclonal antibodies in patient treatment can interfere with recognition of target antigens by this reagent. This should be considered when analyzing samples from patients treated in this fashion. BD Biosciences has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.
- Use of this reagent for diagnostic evaluation of hematologic disorders should be performed in the context of a thorough immunophenotypic analysis including other relevant markers.
- Use of BD OneFlow™ LST requires experience with leukemia and lymphoma immunophenotyping and classification. The results should be interpreted by a pathologist, or equivalent professional, in conjunction with other clinical or laboratory findings.
- BD OneFlow™ LST has not been tested on specimens from patients with minimal residual disease (MRD).
- Avoid using potentially compromised specimens, including clotted, hemolyzed, frozen, or refrigerated specimens.

10. PERFORMANCE CHARACTERISTICS

BD FACSLyric™ Flow Cytometer

Precision studies for the reproducibility and repeatability of BD OneFlow™ LST were performed at BD Research Centre Ireland.

Reproducibility and repeatability (BD FACSLyric™ flow cytometer)

A 5-day study was performed at one site to assess the reproducibility and repeatability of BD OneFlow™ LST using control material. Estimates of precision were determined across three BD FACSLyric™ flow cytometers and three operators by acquiring BD Multi-Check™ Control, stained in duplicate by each operator using three lots of BD OneFlow™ LST. Two separate runs were performed by each operator on each of the 5 tested days.

Nine cell populations were identified as being a percentage of the cell populations indicated in the following tables. The tables present the mean, standard deviation (SD), coefficient of variation (%CV), and the upper 95% confidence limit (CL) for reproducibility (operator/instrument-to-operator/instrument, lot-to-lot, run-to-run, and day-to-day reproducibility) and repeatability (within-run precision) for each subset percentage.

Table 3 Reproducibility of subset percentages

Subset	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	99.78	0.13	0.15	0.14	0.15
Lymphocytes (%Leukocytes)	36.56	0.94	1.08	2.58	2.90
T cells (%Lymphocytes)	68.48	1.11	1.26	1.61	1.81
$CD4^+CD8^-$ cells (%T cells)	66.05	0.30	0.34	0.45	0.50

Table 3 Reproducibility of subset percentages

Subset	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
$CD8^+CD4^-$ cells (%T cells)	29.66	0.39	0.44	1.31	1.47
B cells (%Lymphocytes)	12.99	0.34	0.39	2.62	2.95
NK cells (%Lymphocytes)	17.48	1.01	1.16	5.80	6.53
IgK cells (%B cells)	62.57	1.11	1.27	1.78	2.00
IgL cells (%B cells)	33.57	1.53	1.75	4.56	5.12

Table 4 Repeatability of subset percentages

Subset	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	99.78	0.04	0.05	0.04	0.05
Lymphocytes (%Leukocytes)	36.56	0.55	0.63	1.50	1.69
T cells (%Lymphocytes)	68.48	0.47	0.54	0.69	0.78
$CD4^+CD8^-$ cells (%T cells)	66.05	0.35	0.40	0.53	0.59
$CD8^+CD4^-$ cells (%T cells)	29.66	0.35	0.40	1.18	1.32
B cells (%Lymphocytes)	12.99	0.24	0.28	1.88	2.11
NK cells (%Lymphocytes)	17.48	0.34	0.38	1.93	2.16
IgK cells (%B cells)	62.57	1.09	1.25	1.75	1.96
IgL cells (%B cells)	33.57	1.16	1.33	3.47	3.89

Method comparison (BD FACSLyric™ flow cytometer)

A method comparison study between the BD OneFlow™ system on the BD FACSLyric™ flow cytometer (Investigational Method) and the BD OneFlow™ system on the BD FACSCanto™ II flow cytometer (Comparator Method) was performed at 4 clinical sites. The BD OneFlow™ system on BD FACSLyric™ comprises BD® CS&T Beads, BD® FC Beads 7-Color Kit, BD® FC Beads 5-Color Kit, and BD OneFlow™ LST acquired on a 10-color BD FACSLyric™ flow cytometer (4-Blue 3-Red 3-Violet) using BD FACSuite™ Clinical application v1.3† and the OneFlow LST assay. The BD OneFlow™ reference system on BD FACSCanto™ II comprises BD FACSDiva™ CS&T IVD Beads, BD OneFlow™ Setup Beads, BD® FC Beads 8-Color Kit for BD OneFlow™ Assays, and BD OneFlow™ LST acquired on a BD FACSCanto™ II flow cytometer (4-2H-2V) using BD FACSDiva™ software v8.0.2 and the OneFlow LST template. A total of 98 PB specimens, 26 BM specimens and 16 LN specimens were enrolled in the study. Specimens were collected in the anticoagulants shown. See Table 5.

Table 5 Anticoagulants used to collect specimens

	Anticoagulant	
Specimen type	**EDTA**	**Heparin**
PB	72	26
BM	23	3

† A regression study was performed, demonstrating equivalence between BD FACSuite™ Clinical application v1.3 and v1.4.

For PB, BM, and LN specimens, the first wash step was started within 23, 24, or 23 hours of collection, respectively. Stained PB, BM, and LN samples were acquired within 50, 48, or 31 minutes of final resuspension, respectively. Samples were identified as being "Abnormal" (abnormal for B cells, T cells, or NK cells) or "Normal" (normal for T cells, B cells, and NK cells) using the two systems, and compared.

Agreement was calculated as follows:

Overall % agreement = ((a+d)/(a+b+c+d))×100
Positive % agreement = (a/(a+c))×100
Negative % agreement = (d/(d+b))×100

wherein,

a = number of samples "Abnormal" for both systems,
b = number of samples "Abnormal" on the BD FACSLyric™ flow cytometer but "Normal" on the BD FACSCanto™ II flow cytometer,
c = number of samples "Normal" on the BD FACSLyric™ flow cytometer but "Abnormal" on the BD FACSCanto™ II flow cytometer,
d = number of samples "Normal" for both systems.

The results for all cell types are shown in Table 6.

Table 6 Agreement for all cells being "Abnormal" or "Normal"

		Comparator method (BD FACSCanto™ II flow cytometer		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric™ flow cytometer)	Abnormal	63	0	63
	Normal	0	77	77
	Total	63	77	140

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for “Abnormal” (abnormal for B cells, T cells, and NK cells) is 100%. The negative agreement for “Normal” (normal for T cells, B cells, and NK cells) is 100%.

The results for B cells are shown in Table 7.

Table 7 Agreement for B cells being “Abnormal” or “Normal”

		Comparator method (BD FACSCanto™ II flow cytometer		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric™ flow cytometer)	Abnormal	52	0	52
	Normal	0	88	88
	Total	52	88	140

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for “Abnormal” (abnormal for B cells) is 100%. The negative agreement for “Normal” (normal for B cells) is 100%.

The results for T cells are shown in Table 8.

Table 8 Agreement for T cells being “Abnormal” or “Normal”

		Comparator method (BD FACSCanto™ II flow cytometer		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric™ flow cytometer)	Abnormal	9	0	9
	Normal	0	131	131

Table 8 Agreement for T cells being "Abnormal" or "Normal"

		Comparator method (BD FACSCanto™ II flow cytometer		
		Abnormal	Normal	Total
	Total	9	131	140

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for T cells) is 100%. The negative agreement for "Normal" (normal for T cells) is 100%.

The results for NK cells are shown in Table 9.

Table 9 Agreement for NK cells being "Abnormal" or "Normal"[a]

		Comparator method (BD FACSCanto™ II flow cytometer		
		Abnormal	Normal	Total
Investigational method (BD FACSLyric™ flow cytometer)	Abnormal	2	0	2
	Normal	0	138	138
	Total	2	138	140

a. NK cell malignancies are rare, therefore the expected number of samples is very low.

Overall % agreement is 100%. The lower 95% confidence limit is 97.88%.

The positive agreement for "Abnormal" (abnormal for NK cells) is 100%. The negative agreement for "Normal" (normal for NK cells) is 100%.

Equivalency (BD FACSLyric™ flow cytometer)

A quantitative assessment of the indicated cell populations, expressed as a percentage of another cell population, was performed for each evaluable specimen enrolled in the method comparison study. Specimens were analyzed using the BD OneFlow™ system on the BD FACSLyric™ flow cytometer and the BD FACSCanto™ II flow cytometer as described previously.

The mean bias for the percentages of the indicated cell populations determined on the BD FACSLyric™ flow cytometer versus the BD FACSCanto™ II flow cytometer was calculated. See Table 10.

Table 10 Summary of mean bias for subset percentages

Population	No. of samples	Mean bias	Lower 95% CL of mean bias	Upper 95% CL of mean bias
Leukocytes (%SSC singlets)	134	–0.27	–0.73	0.19
Lymphocytes (%Leukocytes)	134	–0.63	–1.2	–0.06
T cells (%Lymphocytes)	134	–0.17	–0.58	0.24
$CD4^+CD8^-$ cells (%T cells)	134	–0.53	–1.03	–0.04
$CD8^+CD4^-$ cells (%T cells)	134	0.2	–0.41	0.81
B cells (%Lymphocytes)	134	–0.26	–0.72	0.19
$sIg\kappa^+$ cells (%B cells)	134	–1.19	–2.1	–0.29
$sIg\lambda^+$ cells (%B cells)	134	0.7	0.02	1.38
NK cells (%Lymphocytes)	134	0.45	–0.07	0.98

The results of the method comparison and equivalency studies indicate that the two systems are substantially equivalent.

BD FACSCanto™ II Flow Cytometer

Precision studies for the reproducibility and repeatability of the BD OneFlow™ LST were performed at BD Biosciences laboratories in San Jose, CA.

Reproducibility (BD FACSCanto™ II flow cytometer)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto™ II flow cytometers. For each run, duplicate samples of BD Multi-Check™ Control were stained using three lots of BD OneFlow™ LST by each operator, and then acquired and analyzed using the OneFlow LST template in BD FACSDiva™ software. Nine cell populations were identified as being a percentage of the cell populations indicated in Table 11. The reproducibility of the subset percentages was calculated for each cell population. Reproducibility comprises four components: operator/instrument-to-operator/instrument, lot-to-lot, run-to-run, and day-to-day reproducibility.

Table 11 Reproducibility of subset percentages

Population	Mean	SD[a]	Upper 95% CL[b] of SD	%CV[c]	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.0	0.01	0.03	0.01	0.03
Lymphocytes (%Leukocytes)	37.9	0.5	1.1	1.2	2.9
T cells (%Lymphocytes)	70.3	0.2	0.3	0.3	0.5
$CD4^+CD8^-$ cells (%T cells)	63.4	0.5	1.5	0.8	2.4
$CD8^+CD4^-$ cells (%T cells)	25.1	1.1	3.2	4.4	12.8
B cells (%Lymphocytes)	14.8	0.2	1.0	1.5	6.5
NK cells (%Lymphocytes)	14.8	0.3	0.8	1.9	5.6
$smIg\kappa^+$ cells (%B cells)	59.4	0.2	3.1	0.3	5.2
$smIg\lambda^+$ cells (%B cells)	40.6	0.2	2.8	0.4	6.8

a. SD = Standard deviation
b. CL = Confidence limit
c. %CV = Coefficient of variation

Repeatability (BD FACSCanto™ II flow cytometer)

Two operators performed two separate runs per day over a period of eight days, alternating the runs on two BD FACSCanto™ II flow cytometers. For each run, duplicate samples of BD Multi-Check™ Control were stained using three lots of BD OneFlow™ LST by each operator, and then acquired and analyzed using the OneFlow LST template in BD FACSDiva™ software. Nine cell populations were identified as being a percentage of the cell populations indicated in Table 12. The within-run precision (tube-to-tube repeatability) of the subset percentages was calculated for each of the cell populations.

Table 12 Repeatability of subset percentages

Population	Mean	SD	Upper 95% CL of SD	%CV	Upper 95% CL of %CV
Leukocytes (%SSC Singlets)	100.0	0.03	0.03	0.03	0.03
Lymphocytes (%Leukocytes)	37.9	0.5	0.5	1.3	1.4
T cells (%Lymphocytes)	70.3	0.4	0.4	0.5	0.6
$CD4^+CD8^-$ cells (%T cells)	63.4	0.5	0.6	0.8	0.9
$CD8^+CD4^-$ cells (%T cells)	25.1	0.7	0.8	3.0	3.2
B cells (%Lymphocytes)	14.8	0.3	0.3	1.8	2.0
NK cells (%Lymphocytes)	14.8	0.3	0.3	2.1	2.3
$smIg\kappa^+$ cells (%B cells)	59.4	0.8	0.8	1.3	1.4
$smIg\lambda^+$ cells (%B cells)	40.6	0.8	0.8	1.9	2.1

Method comparison (BD FACSCanto™ II flow cytometer)

A side-by-side comparison study between the BD OneFlow™ LST system on the BD FACSCanto™ II flow cytometer and the EuroFlow LST system on the BD FACSCanto™ II flow cytometer was performed at 3 external clinical sites. The BD OneFlow™ LST system comprises BD OneFlow™ Setup Beads, BD® FC Beads for compensation, and the BD OneFlow™ LST reagent. The EuroFlow LST reference system comprises Sphero™ Rainbow calibration particles (8 peaks), single color stained cells plus BD® Multicolor CompBeads for compensation, and the EuroFlow LST reagent cocktail. Both methods used BD FACSDiva™ CS&T IVD beads to perform instrument quality control. Abnormal mature lymphocyte populations from 81 patients with B-cell malignancies, 35 patients with T-cell malignancies, and 6 patients with NK-cell malignancies were identified using the two systems, and compared. In addition, 9 samples were identified as being from other lineages, for example, plasma cell disorders or bi-phenotypic samples. Cell populations from 76 negative samples, including 19 healthy donors, were identified using the two systems. A total of 123 PB specimens, 53 BM specimens, and 31 fresh LN specimens were enrolled in the study. PB and BM specimens were stained within 24 hours of collection. LN specimens were stained within 6 hours of collection. All stained samples were acquired within 1 hour of staining. Samples were identified as being "Follow-up needed" or "No follow-up needed" using the two systems, and compared.

Agreement was calculated as follows:

Overall % agreement = ((a+d)/(a+b+c+d))×100

wherein,

a = number of samples "Follow-up needed" for both systems,

b = number of samples "Follow-up needed" for the BD OneFlow™ system but "No follow-up needed" for the EuroFlow system,

c = number of samples "No follow-up needed" for the BD OneFlow™ system but "Follow-up needed" for the Euroflow system, and

d = number of samples "No follow-up needed" for both systems.

The results for all cell types are shown in Table 13.

Table 13 Agreement for all cells being "Follow-up needed" or "No follow-up needed"

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow™ LST)	Follow-up needed	131	0	131
	No follow-up needed	0	76	76
	Total	131	76	207

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

The results for T cells are shown in Table 14.

Table 14 Agreement for T cells being "Follow-up needed" or "No follow-up needed"

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow™ LST)	Follow-up needed	35	0	35
	No follow-up needed	0	172	172
	Total	35	172	207

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

The results for B cells are shown in Table 15.

Table 15 Agreement for B cells being "Follow-up needed" or "No follow-up needed"

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow™ LST)	Follow-up needed	81	0	81
	No follow-up needed	0	126	126
	Total	81	126	207

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

The results for NK cells are shown in Table 16.

Table 16 Agreement for NK cells being "Follow-up needed" or "No follow-up needed"[a]

		Comparator method (Euroflow LST cocktail)		
		Follow-up needed	No follow-up needed	Total
Investigational method (BD OneFlow™ LST)	Follow-up needed	6	0	6
	No follow-up needed	0	201	201
	Total	6	201	207

a. NK cell malignancies are rare, therefore the expected number of samples is very low.

Overall % agreement is 100%. The lower 95% confidence limit is 98.6%.

Equivalency (BD FACSCanto™ II flow cytometer)

Peripheral blood, bone marrow, and lymph node specimens collected at 3 external clinical laboratories were obtained from patients with T-cell, B-cell, or NK-cell abnormalities, or with no hematological abnormalities. Specimens were analyzed using the BD OneFlow™ LST system and the EuroFlow LST system described previously.

The bias for leukocytes identified as being a percentage of SSC singlets is summarized in Table 17.

Table 17 Summary of bias for Leukocytes (%SSC singlets)

Population	No. of samples	Average bias	Lower 95% CL of average bias	Upper 95% CL of average bias
Leukocytes (%SSC singlets)	207	–1.1%	–1.7%	–0.6%

The remaining eight cell populations were identified as being a percentage of the cell populations indicated in Table 18. Deming regression statistics indicate that the results obtained using the two systems are substantially equivalent.

Table 18 Equivalency of the BD OneFlow™ system to the EuroFlow system

Population	No. of samples	Intercept	Slope	Lower 95% CL of slope	Upper 95% CL of slope
Lymphocytes (%Leukocytes)	207	–0.68	1.01	1.00	1.02
T cells (%Lymphocytes)	207	1.07	0.99	0.98	1.00
$CD4^+CD8^-$ cells (%T cells)	207	–0.64	1.01	1.00	1.02
$CD8^+CD4^-$ cells (%T cells)	207	–0.45	1.00	0.99	1.01
B cells (%Lymphocytes)	207	–0.01	1.00	0.99	1.00
NK cells (%Lymphocytes)	207	0.81	0.92	0.84	1.00
$smIg\kappa^+$ cells (%B cells)	206[a]	2.24	1.00	0.97	1.02
$smIg\lambda^+$ cells (%B cells)	206[a]	–2.04	1.00	0.98	1.03

a. One patient specimen had 0 and 2 B-cell events for the two systems, respectively. Since smIgκ and smIgλ are defined as a percentage of B-cell events in this study, they could not be defined in one system, and therefore could not be included in the quantitative analysis for smIgκ and smIgλ for that specimen.

11. TROUBLESHOOTING

Problems with cell preparation or staining

Problem	Possible Cause	Solution
The resolution between debris and lymphocytes is poor.	Specimen was poorly lysed.	Prepare and stain another specimen.
	Specimen is of poor quality.	Check cell viability.
	Specimen is too old.	Obtain a new specimen and stain it immediately.
Staining is dim or fading.	Cell concentration was too high at the staining step.	Check the cell concentration and adjust as needed.
	Washed specimen was not stained within 30 minutes of the last wash.	Repeat staining with a freshly prepared specimen.
	BD OneFlow™ LST was exposed to light for too long.	Repeat staining with a new tube of BD OneFlow™ LST.
	Stained cells were stored too long before acquiring them.	Repeat staining with a fresh specimen and acquire it promptly.
Few or no cells are recorded.	Cell concentration was too low.	Centrifuge specimen and resuspend it at a higher concentration. Repeat staining and acquisition.
	Cytometer is malfunctioning.	Troubleshoot the instrument. See the cytometer IFU for more information.

Problems using BD OneFlow LST on BD FACSLyric flow cytometers:

Problem	Possible Cause	Solution
Not enough cells of interest are acquired.	Cell concentration was too low.	Centrifuge specimen and resuspend it at a higher concentration. Repeat staining and acquisition.
	The default setting of 100,000 events acquired is too low.	Change the number of events acquired. Repeat staining and acquisition. See the *BD OneFlow™ LST Application Guide For the BD FACSLyric™ Flow Cytometer*.

Problem	Possible Cause	Solution
The FSC-A vs SSC-A dot plot is abnormal.	Cytometer needs adjusting.	Contact BD Biosciences.
The csv file and report are not exported automatically.	The reagent lot number and expiration date were not added to the Library.	1. Add the reagent lot number and expiration date to the Library. 2. Export the csv file and the report PDF manually. See the *BD FACSLyric™ Clinical System Instructions For Use*.

Problems using BD OneFlow LST on BD FACSCanto II flow cytometers:

Problem	Possible Cause	Solution
The resolution between debris and lymphocytes is poor.	Instrument settings are inappropriate.	Follow proper instrument setup procedures. See the *Instrument Setup Guide for BD OneFlow™ Assays*.
Some of the dot plots are dimmed.	FSC-H and SSC-H were not selected when the application settings were created.	Check that FSC-H and SSC-H are selected on the **Parameters** tab of the **Inspector**.
The barcode on the BD OneFlow™ LST tube label cannot be scanned.	The barcode on the tube label has been compromised.	Scan the barcode on the BD OneFlow™ LST pouch label into the **Product ID** keyword field in the **Experiment Layout**. Next, manually enter a semicolon (;) followed by the six-digit tube-specific ID, found adjacent to the barcode on the tube label, after the last digit of the barcode.
The dot plots on the worksheets are missing or the dot plots do not have gates.	The template did not import correctly.	1. Close the current experiment. 2. Create a new experiment. 3. Re-import the BD OneFlow™ LST template.

Problem	Possible Cause	Solution
Some of the keywords are missing from the statistics box in the analysis worksheet.	BD FACSDiva™ software did not import all of the keywords into the panel template.	1. In the **Browser**, set the current tube pointer to the tube that you are analyzing. 2. Navigate to the analysis worksheet. 3. Right-click the statistics box and select **Edit Stats View**. 4. In the **Header** tab, select the **All** checkbox. 5. Click **OK**.
The statement, **For in vitro diagnostic use**, does not appear in the footer of the analysis worksheet when it is printed.	The paper margins in the printer settings were changed.	1. From the BD FACSDiva™ software menu bar, select **File > Page Setup**. 2. Ensure that all of the margins are set to 2.54 cm or 1 inch, depending on your default standards. 3. Click **OK**.

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.

TRADEMARKS

REFERENCES

1 van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.

2 Kalina T, Flores-Montero J, van der Velden VH, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia.* 2012;26:1986-2010.

3 Bernard A, Boumsell L, Hill C. Joint report of the first international workshop on human leucocyte differentiation antigens by the investigators of the participating laboratories. In: Bernard A, Boumsell L, Dausset J, Milstein C, Schlossman SF, eds. *Leucocyte Typing.* New York, NY: Springer-Verlag; 1984:9-108.

4 Kubagawa H, Gathings WE, Levitt D, Kearney JF, Cooper MD. Immunoglobulin isotype expression of normal pre-B cells as determined by immunofluorescence. *J Clin Immunol.* 1982;2:264-269.

5 Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J Immunol.* 1986;136:4480-4486.

6 Ritz J, Trinchieri G, Lanier LL. NK-cell antigens: section report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens.* Vol 2. New York, NY: Oxford University Press; 1995:1367-1372.

7 Engleman EG, Warnke R, Fox RI, Dilley J, Benike CJ, Levy R. Studies of a human T lymphocyte antigen recognized by a monoclonal antibody. *Proc Natl Acad Sci USA.* 1981;78:1791-1795.

8 Knowles RW. Immunochemical analysis of the T-cell–specific antigens. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human T Lymphocytes.* Vol 1. New York, NY: Springer-Verlag; 1986:259-288.

9 Ling NR, Maclennan ICM, Mason DY. B-cell and plasma cell antigens: new and previously defined clusters. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:302-335.

10 Nadler LM. B Cell/Leukemia Panel Workshop: Summary and Comments. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human B Lymphocytes*. Vol 2. New York, NY: Springer-Verlag; 1986:3-43.

11 Borst J, van Dongen J, Bolhuis R, et al. Distinct molecular forms of human T cell receptor γ/δ detected on viable T cells by a monoclonal antibody. *J Exp Med*. 1988;167:1625-1644.

12 Ichinohasama R, Miura I, Takahashi T, et al. Peripheral CD4+ CD8– γδ T cell lymphoma: a case report with multiparameter analyses. *Hum Pathol*. 1996;27:1370-1377.

13 Cobbold SP, Hale G, Waldmann H. Non-lineage, LFA-1 family, and leucocyte common antigens: new and previously defined clusters. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:788-803.

14 Appendix C. Summary of antibody names, code numbers, and donor laboratories. In: McMichael AJ, Beverley PC, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York, NY: Oxford University Press; 1987:988-993.

15 Rothe G, Schmitz G. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. *Leukemia*. 1996;10:877-895.

16 Stelzer GT, Marti G, Hurley A, McCoy PJ, Lovett EJ, Schwartz A. US-Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: standardization and validation of laboratory procedures. *Cytometry*. 1997;30:214-230.

17 Nicholson JKA, Green TA. Selection of anticoagulants for lymphocyte immunophenotyping: effect of specimen age on results. *J Immunol Methods*. 1993;165:31-35.

18 Paxton H, Bendele T. Effect of time, temperature, and anticoagulant on flow cytometry and hematological values. *Ann NY Acad Sci*. 1993:677:440-443.

19 *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.

20 Centers for Disease Control. Perspectives in disease prevention and health promotion 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.

21 Davis BH, Dasgupta A, Kussick S, Han JY, Estrellado A; on behalf of ICSH/ICCS working group. Validation of cell-based fluorescence assays: practice guidelines from the ICSH and ICCS - part II - preanalytical issues. *Cytometry* Part B 2013;84B:286-290.

22 Stetler-Stevenson M, Greig B, Yuan C. Flow cytometric specimen collection, processing, and reporting. In: Kottke-Marchant K, Davis BH, eds. *Laboratory Hematology Practice*. First Edition. Hoboken, NJ; Wiley-Blackwell Inc.; 2012:105-114.

23 Stetler-Stevenson M, Ahmad E, Barnett D, et al. *Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells; Approved Guideline—Second Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document H43-A2.

HISTORY

Revision	Date	Change made
23-15586-00	7/2014	Initial release
23-15586-01	12/2016	Revised for new BD OneFlow™ LST template.
23-15586-02	5/2020	Revised to support using the product on BD FACSLyric™ flow cytometers.
23-15586-03	1/2021	Revised to support using the product with the BD FACS™ Universal Loader.

CONTACT INFORMATION

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, CA 95131 USA

Benex Limited
Pottery Road, Dun Laoghaire
Co. Dublin, Ireland
Tel +353.1.202.5222
Fax +353.1.202.5388

BD Biosciences
European Customer Support
Tel +32.53.720.600
help.biosciences@bd.com

Australian and New Zealand
Distributors:

Becton Dickinson Pty Ltd.
66 Waterloo Rd
Macquarie Park NSW 2113
Australia

Becton Dickinson Limited
14B George Bourke Drive
Mt Wellington, Auckland 1060
New Zealand

For technical support, contact:

bdbiosciences.com
ClinicalApplications@bd.com

BD CD81 (JS-81)

Monoclonal Antibodies Detecting Human Antigens

Form	Catalog number
APC-H7	656647

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include:

- Studies of B-cell proliferation[1]
- Research on antibody deficiency[2]
- Research on the hepatitis C virus receptor[3,4]

Description

Specificity

The CD81 antibody recognizes a 26-kilodalton (kDa) member of the tetraspanin family (TM4SF).[1] The CD81 antigen is also known as TAPA-1.

Antigen distribution

The CD81 antigen has a very broad cellular distribution, being expressed on cells of hematopoietic, neuroectodermal, and mesenchymal origin. In hematopoietic cells, the CD81 antigen is expressed on B and T lymphocytes, natural killer (NK) cells, thymocytes, eosinophils, germinal center follicular dendritic cells, and to a variable extent on monocytes.[5] The CD81 antigen is not expressed on neutrophils, platelets, or erythrocytes.[5]

The CD81 antibody has been shown to have anti-proliferative effects on different lymphoid cell lines, particularly those derived from large cell lymphomas.[1] The CD81 antigen is involved in cell growth and signal transduction.[6] Immunoprecipitation studies reveal that the CD81 antigen is a component of a multimolecular complex that can include the Leu13 molecule,[7] and the CD19 and CD21 molecules in B cells.[8,9]

Clone

The CD81 antibody, clone JS-81,[5] is derived from the hybridization of P3/NS1/1-Ag4-1 mouse myeloma cells with spleen cells isolated from mice immunized with the Burkitt lymphoma cell line, Ramos.[10]

Composition

The CD81 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following is supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
APC-H7	100	5	50	0.5	100	BSA	CMIT/MIT (3:1)

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

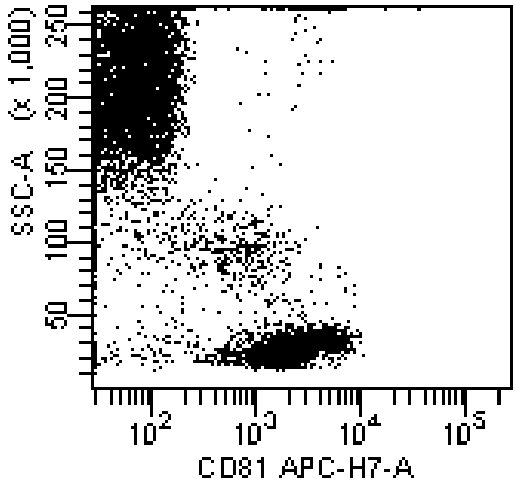
CAUTION Some APC-H7 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

Procedure

Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Representative Data

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 635 nm. Representative data analyzed with a BD flow cytometer is shown in the following plot.



Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Warning

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[11,12] 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.

The APC-H7 conjugate contains a mixture of 0.00236% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one [CMIT/MIT (3:1)], CAS number 55965-84-9. These reagents are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

	Warning
	H317: May cause an allergic skin reaction.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray.

	Warning
	P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection.
Response	P302+P352: IF ON SKIN: Wash with plenty of water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P321: Specific treatment (see supplemental first aid instructions on Safety Data Sheet). P321: Specific treatment (see Safety Data Sheet). P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

References

1. Oren R, Takahashi S, Doss C, Levy R, Levy S. TAPA-1, the target of an antiproliferative antibody, defines a new family of transmembrane proteins. *Mol Cell Biol.* 1990;10:4007-4015.
2. van Zelm MC, Smet J, Adams B, et al. CD81 gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency. *J Clin Invest.* 2010;120:1265-1274.
3. Flint M, Maidens C, Loomis-Price LD, et al. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. *J Virol.* 1999;73:6235-6244.
4. Petracca R, Falugi F, Galli G, et al. Structure-function analysis of hepatitis C virus envelope-CD81 binding. *J Virol.* 2000;74:4824-4830.
5. Tedder TF, Wagner N, Engel P. CD81 Workshop report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leukocyte Typing V: White Cell Differentiation Antigens.* Vol 1. New York, NY: Oxford University Press; 1995:684-688.
6. Levy S, Todd SC, Maecker HT. CD81 (TAPA-1): a molecule involved in signal transduction and cell adhesion in the immune system. *Ann Rev Immunol.* 1998;16:89-109.
7. Takahashi S, Doss C, Levy S, Levy R. TAPA-1, the target of an antiproliferative antibody, is associated on the cell surface with the Leu-13 antigen. *J Immunol.* 1990;145:2207-2213.

8. Bradbury LE, Kansas GS, Levy S, Evans RL, Tedder TF. The CD19/CD21 signal transducing complex of human B lymphocytes includes the target of antiproliferative antibody-1 and Leu-13 molecules. *J Immunol.* 1992;149:2841-2850.
9. Fearon DT, Carroll MC. Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. *Annu Rev Immunol.* 2000;18:393-422.
10. Pesando JM, Hoffman P, Conrad T. Malignant human B cells express two populations of p24 surface antigens. *J Immunol.* 1986;136:2709-2714.
11. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
12. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

Patents and Trademarks

For US patents that may apply, see bd.com/patents.

Contact Information

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

bdbiosciences.com
ResearchApplications@bd.com

BD CD9 (M-L13)

Monoclonal Antibodies Detecting Human Antigens

Form	Catalog number	Form	Catalog number
FITC	341646	APC	341648
PE	341647	APC-H7	655409
PerCP-Cy5.5	341649	V450	658167

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include:

- Characterization of leukemias and lymphomas[1-3]
- Analysis of hematopoiesis[4]
- Investigation of T-cell activation and apoptosis[5]
- Detection of platelet activation and aggregation[1,6-8]

Description

Specificity

The CD9 antibody recognizes a 24-kilodalton (kDa) cell surface glycoprotein[9-13] belonging to the tetraspanin family.[1] The CD9 antigen is also known as p24, tetraspanin-29 (Tspan-29), motility-related protein-1 (MRP-1), and leucocyte antigen MIC3.[14]

Antigen distribution

The CD9 antigen has a very broad tissue distribution. It is present on basophils, eosinophils, monocytes, pre-B cells, B cells, and various leukemic cell lines (erythroid, myeloid, some T lymphoid, and pre-B lymphoid). It is also found on follicular center cells, sinus histiocytes, macrophages, Kupffer cells, osteoclasts, hepatocytes, bile duct endothelia, renal glomeruli, proximal and distal tubuli, epithelia (intercellular spinous spaces) of skin and mucosa, fibroblasts, connective tissues, endothelia, smooth muscle, cardiac muscle, synovial lining cells, brain white matter, and peripheral nerves.[9-13]

The CD9 antibody provides a co-stimulatory signal to T cells, resulting in a transient activation followed by apoptosis.[5] The CD9 antibody activates platelets, inducing mitogenesis, aggregation,[1,6-8] and p72syk kinase activity.[15] The CD9 antigen associates with integrins and can modulate integrin signaling, cell migration, and cell adhesion.[1,5,9-13,16]

Clone

The CD9 antibody, clone M-L13,[6,7] is derived from the hybridization of mouse myeloma cells with spleen cells isolated from mice immunized with common acute lymphoblastic leukemia (cALL) cells.

Composition

The CD9 antibody is composed of mouse IgG_1 heavy chains and kappa light chains.

Product configuration

The following are supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (µL)	Amount provided (µg)	Total volume (mL)	Concentration (µg/mL)	Stabilizer	Preservative
FITC	50	20	25	1.0	25	Gelatin	0.1% Sodium azide
PE	50	20	25	1.0	25	Gelatin	0.1% Sodium azide
PerCP-Cy5.5	50	20	25	1.0	25	Gelatin	0.1% Sodium azide
APC	100	5	50	0.5	100	Gelatin	0.1% Sodium azide
APC-H7	100	5	50	0.5	100	BSA	CMIT/MIT (3:1)
V450[a]	100	5	50	0.5	100	Gelatin	0.1% Sodium azide

[a] BD Horizon™ V450

CAUTION Some APC-H7 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde or light. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

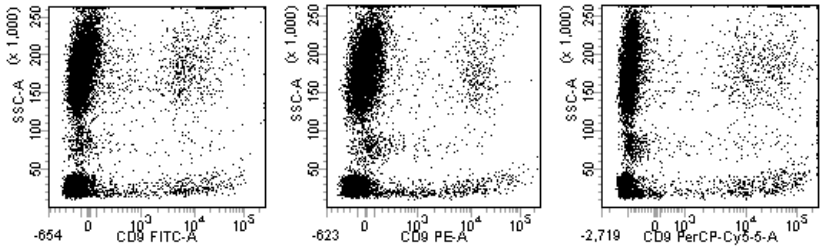
CAUTION Prolonged exposure of cells to paraformaldehyde can lead to increased autofluorescence in the violet channels. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

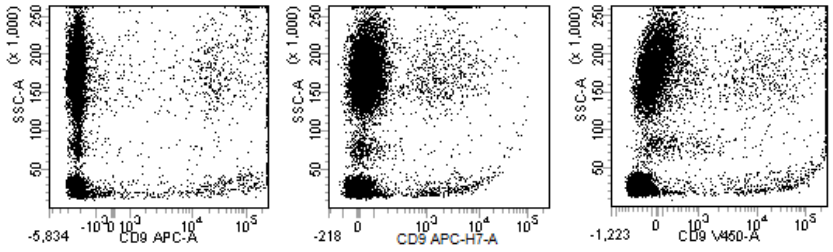
Procedure

Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

Representative Data

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody and gated on lymphocytes. Laser excitation was at 405 nm, 488 nm, or 635 nm. Representative data analyzed with a BD flow cytometer is shown in the following plots.





Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Warning

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[17,18] 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.

The APC-H7 conjugate contains a mixture of 0.00236% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one [CMIT/MIT (3:1)], CAS number 55965-84-9; 5-chloro-2-methyl-4-isothiazolin-3-one [EC number 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC number 220-239-6] (3:1). These reagents are classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

	Warning
	H317: May cause an allergic skin reaction.
Prevention	P261: Avoid breathing dust/fume/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves/protective clothing/eye protection/face protection.
Response	P302+P352: IF ON SKIN: Wash with plenty of water. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362+P364: Take off contaminated clothing and wash it before reuse.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent.

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.

References

1. Maecker HT, Todd SC, Levy S. The tetraspanin superfamily: molecular facilitators. *FASEB J.* 1997;11:428-442.
2. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia.* 2012;26:1908-1975.
3. Weir EG, Cowan K, LeBeau P, Borowitz MJ. A limited antibody panel can distinguish B-precursor acute lymphoblastic leukemia from normal B precursors with four color flow cytometry: implications for residual disease detection. *Leukemia.* 1999;13:558-567.
4. Aoyama K, Oritani K, Yokota T, et al. Stromal cell CD9 regulates differentiation of hematopoietic stem/progenitor cells. *Blood.* 1999;93:2586-2594.
5. Tai XG, Toyooka K, Yashiro Y, et al. CD9-mediated costimulation of TCR-triggered naive T cells leads to activation followed by apoptosis. *J Immunol.* 1997;159:3799-3807.
6. Boucheix C, Benoit P, Krief P, et al. Platelet aggregation induced by CD9 mAbs. Mechanisms and comparison with platelet aggregating properties of mAbs directed against other membrane antigens. In: McMichael AJ, Beverley PCL, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:780-782.
7. Slaper-Cortenbach ICM, Admiraal LG, von dem Borne AEG, van Leeuwen EF, Tetteroo PAT. Epitope specificity and complement-binding studies with monoclonal antibodies (CD9, 10, 24) useful for purging autologous bone-marrow grafts of cALL patients. In: McMichael AJ, Beverley PCL, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:480-484.
8. Zeleznik NJ, Hollingsworth MA, Metzgar RS. Studies of the CD9 antigen defined by monoclonal antibody DU-ALL-1. In: McMichael AJ, Beverley PCL, Cobbold S, et al, eds. *Leucocyte Typing III: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1987:389-392.
9. Johnson GD, MacLennan ICM. Reactivity of the B panel mAb with non-lymphoid constituents of normal tissues. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:187-189.
10. Powling MJ, Cox AD, Higgins J, Hardisty RM, Goodall AH. Phenotypic and functional characterization of pro-aggregatory platelet mAb: subsets of CD9, CD41, and CD36. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:1012-1014.
11. von dem Borne AEG, Admiraal LG, Daams M, Hogervorst F, Modderman PW. Immunocytological evaluation of the mAb of the platelet panel. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:967-970.

12. von dem Borne AEG, Modderman PW. Cluster report: CD9. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:989-990.

13. von dem Borne AEG, Modderman PW, Admiraal LG, Nieuwenhuis HK. Platelet antibodies, the overall results. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:951-966.

14. Zola H, Swart B, Nicholson I, Voss E. *Leukocyte and Stromal Cell Molecules: The CD Markers.* Hoboken, NJ: John Wiley & Sons, Inc.; 2007.

15. Ozaki Y, Satoh K, Kuroda K, et al. Anti-CD9 monoclonal antibody activates p72syk in human platelets. *J Biol Chem.* 1995;270:15119-15124.

16. Berditchevski F, Odintsova E. Characterization of integrin-tetraspanin adhesion complexes: role of tetraspanins in integrin signaling. *J Cell Biol.* 1999;146:477-492.

17. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.

18. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

Patents and Trademarks

For US patents that may apply, see bd.com/patents.

Cy™ is a trademark of GE Healthcare. This product is subject to proprietary rights of GE Healthcare and Carnegie Mellon University, and is made and sold under license from GE Healthcare. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one, return this material, unopened, to BD Biosciences, 155 North McCarthy Boulevard, Milpitas, California 95035, and any money paid for the material will be refunded.

Contact Information

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

bdbiosciences.com
ResearchApplications@bd.com

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

SAFETY DATA SHEET

1. Identification

Product identifier

Product No.:	Product name:	Common name(s), synonym(s)
349524	BD® CellWASH	No data available

Other means of identification
SDS number: 088100210615

Recommended use and restriction on use

Recommended use: Scientific and Industrial laboratory use.
Restrictions on use: None known.

Manufacturer/Importer/Supplier/Distributor Information

Manufacturer

Company Name:	Becton, Dickinson and Company - BD Biosciences
Address:	2350 Qume Drive 95131 San Jose, CA USA
Telephone:	1 877 232 8995 or 1 800 424 9300
Fax:	
Contact Person:	Technical Services
E-mail:	ResearchApplications@bd.com or ClinicalApplications@bd.com

Emergency telephone number: CHEMTREC 1 800 424 9300

2. Hazard(s) identification

Hazard Classification

Not classified

Label Elements

Hazard Symbol: No symbol

Signal Word: No signal word.

Hazard Statement: Not applicable

Precautionary Statements Not applicable

Other hazards which do not result in GHS classification None.

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

3. Composition/information on ingredients

Mixtures

Chemical Identity	Common name and synonyms	CAS number	Content in percent (%)*
Sodium azide (Na(N3))	No data available.	26628-22-8	0 - 0.1%

* All concentrations are percent by weight unless ingredient is a gas. Gas concentrations are in percent by volume.

4. First-aid measures

General information: Get medical attention if symptoms occur.

Ingestion: Call a physician or poison control center immediately. Only induce vomiting at the instruction of medical personnel. Never give anything by mouth to an unconscious person.

Inhalation: Provide fresh air, warmth and rest, preferably in comfortable upright sitting position.

Skin Contact: Wash contact areas with soap and water. Remove contaminated clothing. Launder contaminated clothing before reuse.

Eye contact: Immediately flush with plenty of water for at least 15 minutes. If easy to do, remove contact lenses.

Most important symptoms/effects, acute and delayed

Symptoms: No data available.

Indication of immediate medical attention and special treatment needed

Treatment: No data available.

5. Fire-fighting measures

General Fire Hazards: Extinguish all ignition sources. Avoid sparks, flames, heat and smoking. Ventilate. Use water spray to keep fire-exposed containers cool.

Suitable (and unsuitable) extinguishing media

Suitable extinguishing media: Use fire-extinguishing media appropriate for surrounding materials.

Unsuitable extinguishing media: Not applicable

Specific hazards arising from the chemical: Fire or excessive heat may produce hazardous decomposition products.

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

Special protective equipment and precautions for firefighters

Special fire fighting procedures:	No unusual fire or explosion hazards noted.
Special protective equipment for fire-fighters:	Firefighters must use standard protective equipment including flame retardant coat, helmet with face shield, gloves, rubber boots, and in enclosed spaces, SCBA.

6. Accidental release measures

Personal precautions, protective equipment and emergency procedures:	Contact local authorities in case of spillage to drain/aquatic environment. Ensure suitable personal protection (including respiratory protection) during removal of spillages in a confined area.
Methods and material for containment and cleaning up:	Absorb spillage with suitable absorbent material. Prevent runoff from entering drains, sewers, or streams. See Section 8 of the SDS for Personal Protective Equipment. For waste disposal, see section 13 of the SDS.
Environmental Precautions:	Avoid release to the environment.

7. Handling and storage

Precautions for safe handling:	When using do not eat, drink or smoke. Read and follow manufacturer's recommendations. Use personal protective equipment as required.
Conditions for safe storage, including any incompatibilities:	Store in a cool, dry place. Keep container tightly closed.

8. Exposure controls/personal protection

Control Parameters

Occupational Exposure Limits

Chemical Identity	Type	Exposure Limit Values	Source

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

Sodium azide (Na(N3)) - Vapor. - as hydrazoic acid vapor	CEILING	0.11 ppm 0.3 mg/m3	Canada. Alberta OELs (Occupational Health & Safety Code, Schedule 1, Table 2), as amended (07 2009)
Sodium azide (Na(N3))	CEILING	0.29 mg/m3	Canada. Alberta OELs (Occupational Health & Safety Code, Schedule 1, Table 2), as amended (07 2009)
Sodium azide (Na(N3)) - as NaN3	CEILING	0.29 mg/m3	Canada. British Columbia OELs. (Occupational Exposure Limits for Chemical Substances, Occupational Health and Safety Regulation 296/97, as amended) (07 2007)
Sodium azide (Na(N3)) - Vapor. - as hydrazoic acid vapor	CEILING	0.11 ppm	Canada. British Columbia OELs. (Occupational Exposure Limits for Chemical Substances, Occupational Health and Safety Regulation 296/97, as amended) (07 2007)
Sodium azide (Na(N3)) - as NaN3	CEILING	0.29 mg/m 3	Canada. Manitoba OELs (Reg. 217/2006, The Workplace Safety And Health Act), as amended (03 2011)
Sodium azide (Na(N3)) - as hydrazoic acid vapor	CEILING	0.11 ppm	Canada. Manitoba OELs (Reg. 217/2006, The Workplace Safety And Health Act), as amended (03 2011)
Sodium azide (Na(N3)) - as NaN3	CEV	0.29 mg/m3	Canada. Ontario OELs. (Control of Exposure to Biological or Chemical Agents), as amended (11 2010)
Sodium azide (Na(N3)) - Vapor. - as hydrazoic acid vapor	CEV	0.11 ppm	Canada. Ontario OELs. (Control of Exposure to Biological or Chemical Agents), as amended (06 2015)
Sodium azide (Na(N3)) - as NaN3	Ceiling	0.29 mg/m3	Canada. Saskatchewan OELs (Occupational Health and Safety Regulations, 1996, Table 21), as amended (05 2009)
Sodium azide (Na(N3)) - Vapor. - as hydrazoic acid	Ceiling	0.11 ppm	Canada. Saskatchewan OELs (Occupational Health and Safety Regulations, 1996, Table 21), as amended (05 2009)
Sodium azide (Na(N3))	CEILING	0.11 ppm 0.3 mg/m3	Canada. Quebec OELs. (Ministry of Labor - Regulation Respecting the Quality of the Work Environment), as amended (09 2017)
Sodium azide (Na(N3)) - as NaN3	Ceiling	0.29 mg/m3	US. ACGIH Threshold Limit Values, as amended (12 2010)
Sodium azide (Na(N3)) - as hydrazoic acid vapor	Ceiling	0.11 ppm	US. ACGIH Threshold Limit Values, as amended (12 2010)

Appropriate Engineering Controls

No special requirements under ordinary conditions of use and with adequate ventilation.

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

Individual protection measures, such as personal protective equipment

General information:	Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing to remove contaminants. Discard contaminated footwear that cannot be cleaned.
Eye/face protection:	Wear safety glasses with side shields (or goggles).
Skin Protection	
Hand Protection:	Chemical resistant gloves Suitable gloves can be recommended by the glove supplier. Wash hands after contact.
Skin protection:	Wear a lab coat or similar protective clothing.
Respiratory Protection:	If engineering controls do not maintain airborne concentrations below recommended exposure limits (where applicable) or to an acceptable level (in countries where exposure limits have not been established), an approved respirator must be worn.
Hygiene measures:	Observe good industrial hygiene practices.

9. Physical and chemical properties

Appearance

Physical state:	liquid
Form:	No data available.
Color:	No data available.
Odor:	No data available.
Odor threshold:	No data available.
pH:	No data available.
Melting point/freezing point:	No data available.
Initial boiling point and boiling range:	No data available.
Flash Point:	No data available.
Evaporation rate:	No data available.
Flammability (solid, gas):	No data available.
Upper/lower limit on flammability or explosive limits	
Flammability limit - upper (%):	No data available.
Flammability limit - lower (%):	No data available.
Explosive limit - upper (%):	No data available.
Explosive limit - lower (%):	No data available.
Vapor pressure:	No data available.
Vapor density:	No data available.
Relative density:	No data available.
Solubility(ies)	
Solubility in water:	No data available.

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

Solubility (other):	No data available.
Partition coefficient (n-octanol/water):	No data available.
Auto-ignition temperature:	No data available.
Decomposition temperature:	No data available.
Viscosity:	No data available.

10. Stability and reactivity

Reactivity:	Stable under normal temperature conditions and recommended use.
Chemical Stability:	Material is stable under normal conditions.
Possibility of hazardous reactions:	Not determined.
Conditions to avoid:	Avoid exposure to high temperatures or direct sunlight.
Incompatible Materials:	Metals. Water reactive material.
Hazardous Decomposition Products:	Stable; however, may decompose if heated.

11. Toxicological information

General information: No data on possible toxicity effects have been found.

Information on likely routes of exposure

Ingestion:	No harmful effects expected in amounts likely to be ingested by accident.
Inhalation:	Limited inhalation hazard at normal work temperatures.
Skin Contact:	Negligible irritation to skin at ambient temperatures.
Eye contact:	Elevated temperatures or mechanical action may form vapors, mist, or fumes which may be irritating to the eyes, nose, throat, or lungs.

Symptoms related to the physical, chemical and toxicological characteristics

Ingestion:	No data available.
Inhalation:	No data available.
Skin Contact:	No data available.
Eye contact:	No data available.

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

Information on toxicological effects

Acute toxicity (list all possible routes of exposure)

Oral
Product: No data available.

Dermal
Product: No data available.

Inhalation
Product: No data available.

Repeated dose toxicity
Product: No data available.

Skin Corrosion/Irritation
Product: No data available.

Specified substance(s):
Sodium azide (Na(N3)) Based on available data, the classification criteria are not met.

Serious Eye Damage/Eye Irritation
Product: No data available.

Respiratory or Skin Sensitization
Product: No data available.

Specified substance(s):
Sodium azide (Na(N3)) Not a skin sensitizer.

Carcinogenicity
Product: No data available.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans:
No carcinogenic components identified

US. National Toxicology Program (NTP) Report on Carcinogens:
No carcinogenic components identified

ACGIH Carcinogens:
No carcinogenic components identified

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

Germ Cell Mutagenicity

In vitro
Product: No data available.

In vivo
Product: No data available.

Reproductive toxicity
Product: No data available.

Specific Target Organ Toxicity - Single Exposure
Product: No data available.

Specific Target Organ Toxicity - Repeated Exposure
Product: No data available.

Aspiration Hazard
Product: No data available.

Other effects: No data available.

12. Ecological information

Ecotoxicity:

Acute hazards to the aquatic environment:

Fish
Product: No negative effects on the aquatic environment are known.

Aquatic Invertebrates
Product: No negative effects on the aquatic environment are known.

Chronic hazards to the aquatic environment:

Fish
Product: No negative effects on the aquatic environment are known.

Aquatic Invertebrates
Product: No negative effects on the aquatic environment are known.

Toxicity to Aquatic Plants
Product: No negative effects on the aquatic environment are known.

Persistence and Degradability

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

Biodegradation
Product: Expected to be readily biodegradable.

BOD/COD Ratio
Product: No data available.

Bioaccumulative potential
Bioconcentration Factor (BCF)
Product: No data available.

Partition Coefficient n-octanol / water (log Kow)
Product: No data available.

Mobility in soil: No data available.

Known or predicted distribution to environmental compartments
Sodium azide (Na(N3)) No data available.

Other adverse effects: The product is not expected to be hazardous to the environment.

13. Disposal considerations

General information: Dispose of waste and residues in accordance with local authority requirements.

Disposal instructions: Dispose of waste at an appropriate treatment and disposal facility in accordance with applicable laws and regulations, and product characteristics at time of disposal.

Contaminated Packaging: No data available.

14. Transport information

DOTUN Number:	Not regulated.
UN Proper Shipping Name:	Not regulated.
Transport Hazard Class(es)	
Class:	Not regulated.
Label(s):	Not regulated.
Packing Group:	Not regulated.
Marine Pollutant:	Not regulated.
Limited quantity	Not regulated.
Excepted quantity	Not regulated.

Special precautions for user: Not regulated.

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

IMDG

UN Number:	Not regulated.
UN Proper Shipping Name:	Not regulated.
Transport Hazard Class(es)	
Class:	Not regulated.
Subsidiary risk:	Not regulated.
EmS No.:	Not regulated.
Packing Group:	Not regulated.
Environmental Hazards	
Marine Pollutant:	Not regulated.
Special precautions for user:	Not regulated.

TDG

UN Number	Not regulated.
Proper Shipping Name	Not regulated.
Class	Not regulated.
Packing Group	Not regulated.
Label(s)	Not regulated.
Subsidiary risk label	Not regulated.
Special precautions for user:	Not regulated.

IATA

UN Number:	Not regulated.
Proper Shipping Name:	Not regulated.
Transport Hazard Class(es):	
Class:	Not regulated.
Subsidiary risk:	Not regulated.
Packing Group:	Not regulated.
Environmental Hazards	
Marine pollutant:	Not regulated.
Special precautions for user:	Not regulated.

15. Regulatory information

Canada Federal Regulations
List of Toxic Substances (CEPA, Schedule 1)
Not Regulated

Export Control List (CEPA 1999, Schedule 3)
Not Regulated

National Pollutant Release Inventory (NPRI)
Canada. National Pollutant Release Inventory (NPRI) Substances, Part 5, VOCs with Additional Reporting Requirements
NPRI PT5 Not Regulated

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

Canada. National Pollutant Release Inventory (NPRI) (Schedule 1, Parts 1-4)

NPRI	Not Regulated

Greenhouse Gases

Not Regulated

Controlled Drugs and Substances Act

CA CDSI	Not Regulated
CA CDSII	Not Regulated
CA CDSIII	Not Regulated
CA CDSIV	Not Regulated
CA CDSV	Not Regulated
CA CDSVII	Not Regulated
CA CDSVIII	Not Regulated

Precursor Control Regulations

Not Regulated

International regulations

Montreal protocol

Not applicable

Stockholm convention

Not applicable

Rotterdam convention

Not applicable

Kyoto protocol

Not applicable

16.Other information, including date of preparation or last revision

Issue Date:	05/15/2020
Version #:	1.0
Revision Information:	
Source of information:	European Chemicals Agency (ECHA): Information on Chemicals.

Becton, Dickinson and Company
BD, Franklin Lakes, NJ
07417 USA
www.bd.com

Further Information:	No data available.
Disclaimer:	Disclaimer: The information contained herein has been obtained from various sources and is believed to be correct as of the date issued. However, neither BD nor any of its subsidiaries assumes any liabilities whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability for a particular use of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist. BD provides SDS in electronic form so the information may be more easily accessed. Due to the possibility of errors during transmission, BD makes no representations as to the completeness or accuracy of the information.

BD FACS™ Lysing Solution

Catalog No. 349202

23-1358(14)
2023-04
English

℞ Only CE IVD

1. INTENDED USE

BD FACS™ Lysing Solution is intended for lysing red blood cells for flow cytometric applications. It can be used in both lyse/wash and lyse/no-wash procedures.

2. SUMMARY OF THE TEST

Efficient detection of leukocytes in specimens 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.[1,2,3,4] 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 leukocyte subsets and may help improve assay reproducibility when compared to earlier methods.[5,6,7]

BD FACS™ Lysing Solution is intended for use by laboratory professionals.

Principle of Operation

When the specimen is added to the antibody reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leukocyte surface antigens. The stained samples are then treated with BD FACS™ Lysing Solution, which lyses red blood cells (RBCs) under gentle hypotonic conditions while preserving the leukocytes.

3. REAGENT

Reagent Composition

BD FACS™ Lysing Solution is a proprietary buffered solution containing formaldehyde and diethylene glycol.

Precautions

BD FACS™ Lysing Solution contains 31.34% ethanol, 2,2´-oxybis- (diethylene glycol) (CAS number 111-46-6, EC number 203-872-2), 9.77% formaldehyde (CAS number 50-00-0, EC number 200-001-8), and 3.43% methanol (CAS number 67-56-1, EC number 200-659-6). The lysing solution is classified as hazardous according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Regulation (EC) No 1272/2008, and 29 CFR 1910.1200. Go to regdocs.bd.com/regdocs/sdsSearch to download the Safety Data Sheet.

	Danger
	H302+H312+H332: Harmful if swallowed, in contact with skin or if inhaled. H314: Causes severe skin burns and eye damage. H317: May cause an allergic skin reaction. H335: May cause respiratory irritation. H341: Suspected of causing genetic defects. H350: May cause cancer. H370: Causes damage to organs. H373: May cause damage to organs through prolonged or repeated exposure. US only: H402: Harmful to aquatic life.
Prevention	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 face, hands and any exposed skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P271: Use only outdoors or in a well-ventilated area. P272: Contaminated work clothing should not be allowed out of the workplace. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection.
Response	P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P312: Call a POISON CENTER or doctor/physician if you feel unwell. P303+P361+P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower]. P363: Wash contaminated clothing before reuse. P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing. P310: Immediately call a POISON CENTER/doctor. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P307+P311: IF exposed: Call a POISON CENTER or doctor/ physician. P308+P313: If exposed or concerned: Get medical advice/attention.
Storage	P405: Store locked up.
Disposal	P501: Dispose of contents/container to an approved facility in accordance with local, regional, national and international regulations.

Storage and Handling

- BD FACS™ Lysing Solution (10X) is stable until the expiration date shown on the bottle label when stored as directed.
- The storage temperature is 2–25 °C.
- Do not use this reagent if discoloration occurs or a precipitate forms.

4. 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).

5. SPECIMEN COLLECTION AND PREPARATION

See the instructions for use (IFU) for the reagent you are using for information about specimens supported.

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.

6. PROCEDURE

Reagents and Materials

Reagents and materials provided

BD FACS™ Lysing Solution is provided as 100 mL of a 10X concentrate. After dilution, this volume is sufficient for 2,000 tests when used in lyse/no-wash procedures or for 500 tests when used in lyse/wash procedures.

Reagents and materials required but not provided

- 1X BD FACS™ Lysing Solution, diluted as described
- BD fluorochrome-conjugated antibodies to human leukocyte antigens
- Vortex mixer
- Micropipettor with tips
- Other materials might be required. Refer to the appropriate reagent IFU for more information.

Diluting BD FACS™ Lysing Solution

Dilute the 10X concentrate 1:10 with room temperature (20–25 °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.

Staining the Specimen

Stain the specimen following instructions in the appropriate reagent IFU. Lyse RBCs as directed using diluted (1X) BD FACS™ Lysing Solution.

7. LIMITATIONS

- Samples with nucleated erythrocytes show incomplete lysis of RBCs because BD FACS™ Lysing Solution does not lyse nucleated erythrocytes as efficiently as enucleated RBCs. This may also occur when assaying blood samples from patients with certain hematologic disorders in which RBCs are difficult to lyse, as in myelofibrosis, sickle-cell anemia, thalassemia, and spherocytosis.[7,8]
- BD FACS™ Lysing Solution was developed for use with BD flow cytometers.
- BD FACS™ Lysing Solution was developed using EDTA as the anticoagulant. BD has limited information concerning use of other anticoagulants such as heparin.

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;10:43-55.
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. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Fourth Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.
9. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.

NOTICE

EU Only: Users shall report any serious incident related to the device to the Manufacturer and National Competent Authority.

Outside EU: Contact your local BD representative for any incident or inquiry related to this device.

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.

PATENTS AND TRADEMARKS

For US patents that may apply, see bd.com/patents.

HISTORY

Revision	Date	Changes made
23-1358(13)	2021-11	Updated to meet requirements of Regulation (EU) 2017/746.
23-1358(14)	2023-04	Updated legal manufacturer address. Added EU and Swiss importer addresses and importer symbol. Updated symbols glossary. Added Rx only symbol.

Symbols Glossary

Please refer to product labeling for applicable symbols.

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	Authorised representative in Switzerland
	Date of manufacture
	Use-by date
	Batch code
	Catalogue number
	Serial number
	Sterile
	Sterilized using aseptic processing techniques
	Sterilized using ethylene oxide
	Sterilized using irradiation
	Sterilized using steam or dry heat
	Do not resterilize
	Non-sterile
	Do not use if package is damaged and consult *instructions for use*
	Sterile fluid path
	Sterile fluid path (ethylene oxide)
	Sterile fluid path (irradiation)
	Fragile, handle with care
	Keep away from sunlight
	Keep dry
	Lower limit of temperature
	Upper limit of temperature
	Temperature limit
	Humidity limitation
	Biological risks
	Do not re-use
	Consult *instructions for use* or consult electronic *instructions for use*
	Caution
	Contains or presence of natural rubber latex
	In vitro diagnostic medical device
	Negative control
	Positive control
	Contains sufficient for <*n*> tests
	For IVD performance evaluation only
	Non-pyrogenic
	Patient number
	This way up
	Do not stack

Symbol	Meaning
	Single sterile barrier system
	Contains or presence of phthalate: combination of bis(2-ethylhexyl) phthalate (DEHP) and benzyl butyl phthalate (BBP)
	Collect separately Indicates separate collection for waste of electrical and electronic equipment required.
	CE marking; Signifies European technical conformity
	Device for near-patient testing
	Device for self-testing
	This only applies to US: "Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner."
	Country of manufacture "CC" shall be replaced by either the two letter or the three letter country code.
	Collection time
	Cut
	Peel here
	Collection date
	Keep away from light
	Hydrogen gas is generated
	Perforation
	Start panel sequence number
	End panel sequence number
	Internal sequence number
	<Box #> / <Total Boxes>
	Medical device
	Contains hazardous substances
	Ukrainian conformity mark
	Meets FCC requirements per 21 CFR Part 15
	UL product certification for US and Canada
	Unique device identifier
	Importer
	Place patient label in framed area only
	Magnetic resonance (MR) safe
	Magnetic resonance (MR) conditional
	Magnetic resonance (MR) unsafe
	For use with
	This Product Contains Dry Natural Rubber
	For Export Only
	Instruments

Note: Text layout in symbols is determined by label design.

L006715(08) 2023-03

CONTACT INFORMATION

Becton, Dickinson and Company
BD Biosciences
155 North McCarthy Boulevard
Milpitas, California 95035 USA

EC REP **Becton Dickinson Ireland Ltd.**
Donore Road, Drogheda
Co. Louth, A92 YW26
Ireland

Becton Dickinson Distribution Center NV
Laagstraat 57
9140 Temse, Belgium

CH REP **BD Switzerland Sàrl**
Route de Crassier 17
Business Park Terre-Bonne
Bâtiment A4
1262 Eysins
Switzerland

Becton Dickinson AG
Binningerstrasse 94
4123 Allschwil
Switzerland

BD Biosciences
European Customer Support
Tel +32.53.720.600
help.biosciences@bd.com

Australian and New Zealand Distributors:

Becton Dickinson Pty Ltd.
66 Waterloo Road
Macquarie Park NSW 2113
Australia

Becton Dickinson Limited
14B George Bourke Drive
Mt. Wellington Auckland 1060
New Zealand

Technical Service and Support: In the United States contact BD at 1.877.232.8995 or bdbiosciences.com.
For regions outside the United States, contact your local BD representative or bdbiosciences.com.

ClinicalApplications@bd.com

eifu.bd.com