Monoclonal Antibodies Detecting Human Antigens

BD Multitest™

CD45RA/CD62L/CD3/CD8

Catalog No. 340978 **50 Tests** **20 µL/test**

RESEARCH APPLICATIONS

Research applications include:

- Enumeration of suppressor/cytotoxic naive ($CD3^+CD8^+CD45RA^+CD62L^+$) and suppressor/cytotoxic memory ($CD3^+CD8^+CD45RA^-CD62L^-$, $CD3^+CD8^+CD45RA^-CD62L^+$, or $CD3^+CD8^+CD45RA^+CD62L^-$) T-lymphocyte subsets[1-4]
- Studies of naive/memory T-cell subsets in Hodgkin's disease[2]
- Studies of naive/memory T-cell subsets in HIV/AIDS[3,4]

DESCRIPTION

Specificity

The CD45RA antibody recognizes a 220-kilodalton (kDa) isoform of the leucocyte common antigen (LCA).[5] The CD45RA antigen is a member of the CD45 antigen family that also includes the CD45, CD45RB, and CD45RO antigens.[5]

The CD62L antibody recognizes the leucocyte endothelial cellular adhesion molecule (LECAM) with a molecular weight of 80 kDa.[6] The CD62L antigen belongs to the selectin family of cell adhesion molecules.[7] The CD62L molecule is the human homologue of the murine lymph node homing receptor, MEL 14.[8]

The CD3 antibody recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.[9] This complex is composed of at least six proteins that range in molecular weight from 20–30 kDa.[10] The antigen recognized by the CD3 antibody is noncovalently associated with either α/β or γ/δ TCR (70–90 kDa).[11]

The CD8 antibody recognizes an antigen expressed on the 32 kDa α subunit of a disulfide-linked bimolecular complex.[12] The cytoplasmic domain of the α subunit of the CD8 antigen is associated with the protein tyrosine kinase $p56^{lck}$.[13] The CD8 molecule interacts with class I major histocompatibility complex (MHC) molecules resulting in increased adhesion between the $CD8^+$ T lymphocytes and the target cells.[14-16] Binding of the CD8 molecule to class I MHC molecules enhances the activation of resting T lymphocytes.[14-16]

Antigen distribution

The CD45RA antigen is present on approximately 50% of $CD4^+$ T lymphocytes, approximately 75% of $CD8^+$ T lymphocytes, and on essentially all B lymphocytes and natural killer (NK) lymphocytes.[17] The suppressor/inducer T-lymphocyte subset expresses the phenotype $CD4^+CD45RA^+$.[17] The CD45RA antigen is expressed on naive T lymphocytes; antigen density decreases upon in vitro activation.[18] A selective loss of the $CD4^+CD45RA^+$ subset during active multiple sclerosis has been demonstrated.[19,20]

The antigen recognized by CD62L is present on 61–75% of normal human peripheral blood lymphocytes, 70–80% of E-rosetting cells, 10% of thymocytes, and most

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

12/2014 23-4901-02

B lymphocytes.[21-24] The CD62L antibody also recognizes monocytes, granulocytes, and a subset of NK lymphocytes.[21,25] The CD62L antigen is present on the peripheral blood T-lymphocyte subset that homes to lymph nodes.[8] CD62L identifies regulatory subpopulations of T lymphocytes in both the $CD8^+$ and $CD4^+$ subsets.[21,22,26,27] Approximately 75% of $CD4^+$ T lymphocytes also bear the CD62L antigen.[26] Approximately 60% of $CD8^+$ T lymphocytes are recognized by CD62L.[26]

The CD3 antigen is expressed on 61–85% of normal peripheral blood lymphocytes,[28] 65–85% of thymocytes,[29] and on Purkinje cells in the cerebellum.[30]

The CD8 antigen is expressed on the human suppressor/cytotoxic T-lymphocyte subset ($CD3^+CD8^+$),[31-35] as well as on a subset of NK lymphocytes.[36] The CD8 antigen is expressed on 19–48% of normal peripheral blood lymphocytes[28] and the majority of normal thymocytes.[37]

Clones

The CD45RA antibody, clone L48, is derived from hybridization of Sp2/0 mouse myeloma cells with spleen cells from BALB/c mice immunized with low-buoyant–density human lymphocytes.

The CD62L antibody, clone SK11,[38] is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with peripheral blood T lymphocytes.

The CD3 antibody, clone SK7,[39-41] is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes.

The CD8 antibody, clone SK1, is derived from hybridization of NS-1 mouse myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood T lymphocytes.

Composition

The CD45RA CD3, and CD8 antibodies are each composed of mouse IgG_1 heavy chains and kappa light chains.

The CD62L antibody is composed of mouse IgG_{2a} heavy chains and kappa light chains.

The BD Multitest reagent is supplied as a combination of CD45RA FITC, CD62L PE, CD3 PerCP, and CD8 APC in 1.0 mL of phosphate-buffered saline (PBS) containing bovine serum albumin and 0.1% sodium azide.

PROCEDURE

Visit our website (bdbiosciences.com) or contact your local BD representative for the lyse/no-wash protocol for direct immunofluorescence.

Lyse/No-Wash method for direct immunofluorescence

1. Pipet 20 µL of monoclonal antibody into a labeled tube.

   For absolute counts, use BD Trucount Tubes. Refer to the *BD Trucount Tubes* package insert for more detailed information.

2. Add 50 µL of whole blood.
3. Vortex gently to mix and incubate for 15 minutes in the dark at room temperature (20°C–25°C).
4. Add 1 mL of 1X BD FACS™ lysing solution (Cat. No. 349202) to the tube.

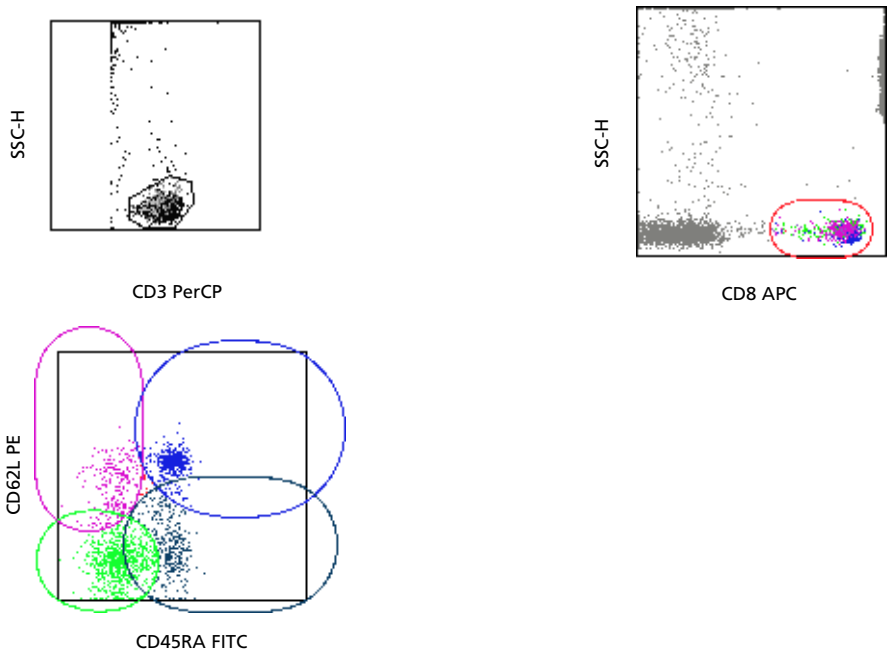
   The volume of BD FACS lysing solution recommended has been optimized for use with this reagent.

5. Vortex gently and incubate for 15–30 minutes in the dark at room temperature.

   If samples are not analyzed immediately, mix thoroughly before analysis.

REPRESENTATIVE DATA

Flow cytometric analysis was performed on peripheral blood leucocytes using a BD FACSCalibur™ flow cytometer with a gate set on the $CD3^+$ lymphocyte fraction. Laser excitation was at 488 nm and 635 nm.



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[42,43] 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. Roederer M, Bigos M, Nozaki T, Stovel RT, Parks DR, Herzenberg LA. Heterogeneous calcium flux in peripheral T cell subsets revealed by five-color flow cytometry using log-ratio circuitry. *Cytometry.* 1995;21:187-196.
2. Watanabe N, De Rosa SC, Cmelak A, et al. Long-term depletion of naive T cells in patients treated for Hodgkin's disease. *Blood*. 1997;90:3662-3672.
3. Rabin RL, Roederer M, Maldonado Y, Petru A, Herzenberg LA, Herzenberg LA. Altered representation of naive and memory CD8 T cell subsets in HIV-infected children. *J Clin Invest*. 1995;95:2054-2060.
4. Roederer M, Dubs JG, Anderson MT, Raju PA, Herzenberg LA, Herzenberg LA. CD8 naive T cell counts decrease progressively in HIV-infected adults. *J Clin Invest*. 1995;95:2061-2066.
5. Cobbold SP, Hale G, Waldmann H. Non-lineage, LFA-1 family, and leucocyte common antigens: new and previously defined clusters. In: McMichael AJ, ed. *Leucocyte Typing III: White Cell Differentiation Antigens*. New York: Oxford University Press; 1987:788-803.

6. Michie SA, Garcia CF, Strickler JG, Dailey MO, Rouse RV, Warnke RA. Expression of the Leu-8 antigen by B-cell lymphomas. *Am J Clin Pathol*. 1987;88:486-490.

7. Bevilacqua M, Butcher E, Furie B, et al. Selectins: a family of adhesion receptors. *Cell*. 1991;67:233.

8. Camerini D, James SP, Stamenkovic I, Seed B. Leu-8/TQ1 is the human equivalent of the Mel-14 lymph node homing receptor. *Nature*. 1989;342:78-82.

9. van Dongen JJM, Krissansen GW, Wolvers-Tettero ILM, et al. Cytoplasmic expression of the CD3 antigen as a diagnostic marker for immature T-cell malignancies. *Blood*. 1988;71:603-612.

10. Brenner MB, Groh V, Porcelli SA, et al. Structure and distribution of the human γ/δ T-cell receptor. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York: Oxford University Press; 1989:1049-1053.

11. Clevers H, Alarcón B, Wileman T, Terhorst C. The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu Rev Immunol*. 1988;6:629-662.

12. Moebius U. Cluster report: CD8. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York: Oxford University Press; 1989:342-343.

13. Rudd CE, Burgess KE, Barber EK, Schlossman SF. Monoclonal antibodies to the CD4 and CD8 antigens precipitate variable amounts of CD4/CD8-associated $p56^{lck}$ activity. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens*. New York: Oxford University Press; 1989:326-327.

14. Gallagher PF, Fazekas de St. Groth B, Miller JFAP. CD4 and CD8 molecules can physically associate with the same T-cell receptor. *Proc Natl Acad Sci, USA*. 1989;86:10044-10048.

15. Anderson P, Blue M-L, Morimoto C, Schlossman SF. Cross-linking of T3 (CD3) with T4 (CD4) enhances the proliferation of resting T lymphocytes. *J Immunol*. 1987;139:678-682.

16. Eichmann K, Jönsson J-I, Falk I, Emmrich F. Effective activation of resting mouse T lymphocytes by cross-linking submitogenic concentrations of the T cell antigen receptor with either Lyt-2 or L3T4. *Eur J Immunol*. 1987;17:643-650.

17. Morimoto C, Letvin N, Distaso J, Aldrich W, Schlossman S. The isolation and characterization of the human suppressor/inducer T-cell subset. *J Immunol*. 1985;134:3762-3769.

18. Serra HM, Krowka JF, Ledbetter JA, Pilarski LM. Loss of CD45R (Lp220) represents a post-thymic T-cell differentiation event. *J Immunol*. 1988;140:1435.

19. Rose LM, Ginsburg AH, Rothstein TL, Ledbetter JA, Clark EA. Selective loss of a subset of T helper cells in active multiple sclerosis. *Proc Natl Acad Sci USA*. 1985;82:7389-7393.

20. Sobel R, Hafler D, Castro E, Morimoto C, Weiner H. The 2H4 (CD45R) antigen is selectively decreased in multiple sclerosis lesions. *J Immunol*. 1988;140:2210-2214.

21. Lanier LL, Engleman EG, Gatenby P, Babcock GF, Warner NL, Herzenberg LA. Correlation of functional properties of human lymphoid cell subsets and surface marker phenotypes using multiparameter analysis and flow cytometry. *Immun Rev*. 1983;74:143.

22. Kansas GS, Wood GS, Fishwild DM, Engleman EG. Functional characterization of human T lymphocyte subsets distinguished by monoclonal Anti-Leu-8. *J Immunol*. 1985;134:2995-3002.

23. Kansas GS, Wood GS, Engleman EG. Maturational and functional diversity of human B lymphocytes delineated with Anti-Leu-8. *J Immunol*. 1985;134:3003-3006.

24. Kanof ME, James SP. Leu-8 antigen expression is diminished during cell activation but does not correlate with effector function of activated T lymphocytes. *J Immunol*. 1988;140:3701-3706.

25. Lanier LL, Loken MR. Human lymphocyte subpopulations identified by using three-color immunofluorescence and flow cytometry analysis: correlation of Leu-2, Leu-3, Leu-7, Leu-8, and Leu-11 cell surface antigen expression. *J Immunol*. 1984;132:151-156.

26. Gatenby PA, Kansas GS, Xian CY, Evans RL, Engleman EG. Dissection of immunoregulatory subpopulations of T lymphocytes within the helper and suppressor sublineages in man. *J Immunol*. 1982;129:1997-2000.

27. Kanof ME, Strober W, James SP. Induction of CD4 suppressor T cells with Anti-Leu-8 antibody. *J Immunol*. 1987;139:49-54.

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

29. 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;137:2501-2507.

30. Garson JA, Beverley PCL, Coakham HB, Harper EJ. Monoclonal antibodies against human T lymphocytes label Purkinje neurones of many species. *Nature*. 1982;298:375-377.

31. Evans RL, Wall DW, Platsoucas CD, et al. Thymus-dependent membrane antigens in man: inhibition of cell-mediated lympholysis by monoclonal antibodies to the T_{H2} antigen. *Proc Natl Acad Sci USA*. 1981;78:544-548.

32. Engleman EG, Benike CJ, Evans RL. Circulating antigen-specific suppressor T cells in a healthy woman: mechanism of action and isolation with a monoclonal antibody. *Clin Res*. 1981;29:365A.

33. Engleman EG, Benike CJ, Glickman E, Evans RL. Antibodies to membrane structures that distinguish suppressor/cytotoxic and helper T lymphocyte subpopulations block the mixed leukocyte reaction in man. *J Exp Med*. 1981;153:193-198.

34. Kotzin BK, Benike CJ, Engleman EG. Induction of immunoglobulin secreting cells in the allogeneic mixed leukocyte reaction: regulation by helper and suppressor lymphocyte subsets in man. *J Immunol*. 1981;127:931-935.

35. Ledbetter JA, Frankel AE, Herzenberg LA. Human Leu T-cell differentiation antigens: Quantitative expression on normal lymphoid cells and cell lines. In: Hämmerling G, Hämmerling U, Kearney J, eds. *Monoclonal Antibodies and T Cell Hybridomas: Perspectives and Technical Notes*. New York: Elsevier/North Holland; 1981:16-22.

36. 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.

37. Wood GS, Warner NL, Warnke RA. Anti–Leu-3/T4 antibodies react with cells of monocyte/macrophage and Langerhans lineage. *J Immunol*. 1983;131:212-216.

38. Diacovo T, Springer T. CD62L (L-selectin) cluster report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens*. New York: Oxford University Press; 1995:1503-1504.

39. Haynes BF. Summary of T-cell studies performed during the Second International Workshop and Conference on Human Leukocyte Differentiation Antigens. In: Reinherz EL, Haynes BF, Nadler LM, Bernstein ID, eds. *Leukocyte Typing II: Human T Lymphocytes*. New York: Springer-Verlag; 1986:3-30.

40. Kan EAR, Wang CY, Wang LC, Evans RL. Noncovalently bonded subunits of 22 and 28 kDa are rapidly internalized by T cells reacted with Anti–Leu-4 antibody. *J Immunol*. 1983;131:536-539.

41. 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*. New York: Springer-Verlag; 1986;1:259-288.

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

43. 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