TECHNICAL DATA SHEET

violetFluor™ 450 Anti-Human CD3 (UCHT1)

Catalog Number: 75-0038

PRODUCT INFORMATION

Contents: violetFluor™ 450 Anti-Human CD3 (UCHT1)

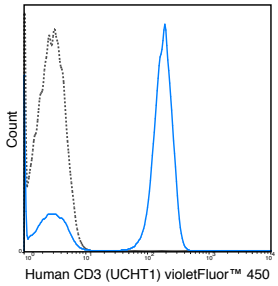
Isotype: Mouse IgG1, kappa

Concentration: 5 uL (0.5 ug)/test

Clone: UCHT1

Reactivity: Human

Formulation: 10 mM NaH2PO4, 150 mM NaCl, 0.09% NaN3, 0.1% gelatin, pH7.2



Human peripheral blood lymphocytes were stained with 5 uL (0.5 ug) violetFluor™ 450 Anti-Human CD3 (75-0038) (solid line) or 0.5 ug violetFluor™ 450 Mouse IgG1 isotype control (dashed line).

DESCRIPTION

The UCHT1 antibody is specific for human CD3e, also known as CD3 epsilon, a 20 kDa subunit of the T cell receptor complex, along with CD3 gamma and CD3 delta. These integral membrane protein chains assemble with additional chains of the T cell receptor (TCR), as well as CD3 zeta chain, to form the T cell receptor – CD3 complex. Together with co-receptors CD4 or CD8, the complex serves to recognize antigens bound to MHC molecules on antigen-presenting cells. These interactions promote T cell receptor signaling (T cell activation), inducing cell proliferation, differentiation, production of cytokines or activation-induced cell death. CD3 is differentially expressed during thymocyte-to-T cell development and on all mature T cells.The UCHT1 antibody is a widely used phenotypic marker for human T cells. In addition, binding/cross-linking of UCHT1 antibody to CD3e can induce cell activation. A recent publication of the crystal structure of a CD3e- antibody complex provides insight as to the action of commonly used agonist antibodies, as well as specific epitope-binding data for the human CD3 antibodies UCHT1 and OKT3 (Fernandes, R.A. et al. 2012. J. Biol. Chem. 287: 13324-13335). UCHT1 antibody reacts with both surface-expressed and intracellular CD3e protein, in contrast to an alternative human CD3 clone, HIT3a, which will stain only the extracellular (membrane-expressed) CD3e protein. Also, the UCHT1 antibody is reported to be cross-reactive with chimpanzee and has been used for phenotypic analysis of expression by flow cytometry; however the antibody is reported to be unsuitable for induction of T cell activation in this species (Bibollet-Ruche et al. 2009. J. Virol. 82: 10271-10278).

PREPARATION & STORAGE

This monoclonal antibody was purified from tissue culture supernatant via affinity chromatography. The purified antibody was conjugated under optimal conditions, with unreacted dye removed from the preparation. It is recommended to store the product undiluted at 4°C, and protected from prolonged exposure to light. Do not freeze.

APPLICATION NOTES

This antibody preparation has been pre-titrated and quality-tested for flow cytometry using an appropriate cell type. The antibody has been diluted for use at 5 uL per test, defined as the amount of antibody that will stain a cell sample in a final volume of approximately 100 uL. The number of cells within a sample should be determined empirically, but typically ranges between 1x10e5 to 1x10e8 cells.

violetFluor™ 450 dye is excited by the violet (405 nm) laser and has a peak emission of 450 nm. The most common band pass filters for this dye are 440/40 or 450/50. violetFluor™ 450 can be used as an alternative for Pacific Blue®, BD Horizon™ V450 or eFluor® 450.

REFERENCES

Harris SJ, Parry RV, Foster JG, Blunt MD, Wang A, Marelli-Berg F, Westwick J, and Ward SG. Apr. 2011. J. Immunol. 186: 4936-4945. (in vitro activation). Beriou G, Bradshaw EM, Lozano E, Costantino CM, Hastings WD, Orban T, Elyaman W, Khoury SJ, Kuchroo VK, Baecher-Allan C, and Hafler DA. 2010. J. Immunol. 185: 46-54. (in vitro activation). Soto PC, Stein LL, Hurtado-Ziola N, Hedrick SM, and Varki A. 2010. J. Immunol. 184: 4185-4195. (Flow cytometry – Chimpanzee). Edelbauer M, Datta D, Vos IHC, Basu A, Stack MP, Reinders MEJ, Sho M, Calzadilla K, Ganze P, and Briscoe DM. 2010. Blood. 116:1980-1989. (Immunohistochemistry – acetone fixed, frozen sections; Immunofluoresence microscopy). Varghese JC and Kane KP. 2008. J. Immunol. 181: 6002-6009. (in vitro activation). Mack CL, Tucker RM, Sokol RJ, Darrer FM, Kotzin BL, Whitington PF and Miller SD. 2004. Pediatr. Res. 56(1):79-87. (Immunohistochemistry – frozen tissue). Sakkas LI, Scanzello C, Johanson N, Burkholder J, Mitra A, Salgame P, Katsetos CD, and Platsoucas CD. 1998. Clin. Diagn. Lab. Immun. 5:430. (Immunohistochemistry – acetone fixed, frozen sections). Salmeron A, Sanchez-Madrid F, Ursa MA, Fresno M, and Alarcon B. 1991. J. Immunol. 147:3047-3052. (Immunoprecipitation). Van Dongen JJ, Krissansen GW, Wolvers-Tettero IL, Comans-Bitter WM, Adriaansen HJ, Hooijkaas H, van Wering ER, and Terhorst C. 1988. Blood. 71: 603-612. (Western Blot).

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