Product Data Sheet

Brilliant Violet 750™ anti-human CD28

Catalog # / 2114850 / 100 tests

Size: 2114845 / 25 tests

Clone: CD28.2

Isotype: Mouse IgG1, κ **Immunogen:** Human CTL line

Reactivity: Human, Non-human primate, Other

Preparation: The antibody was purified by affinity

chromatography and conjugated with Brilliant Violet 750™ under optimal

conditions.

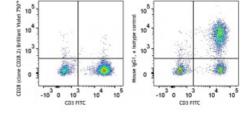
Formulation: Phosphate-buffered solution, pH 7.2,

containing 0.09% sodium azide and

BSA (origin USA)

Workshop Number: V-CD28.05

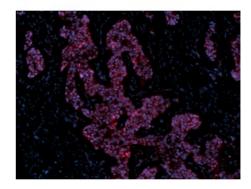
Concentration: Lot-specific



Human peripheral blood lymphocytes were stained with CD3 FITC and CD28 (clone CD28.2) Brilliant Violet 750™ (left) or mouse IgG1, κ Brilliant Violet 750™ isotype control (right).

Applications:

Applications: Flow Cytometry



Formalin-fixed paraffin-embedded human breast cancer tissue slices were deparaffinized and rehydrated. Antigen retrieval was done with Tris-Buffered Saline 1X (1.0 M, pH 7.4) at 95°C for 40 minutes, washed with PBS/0.05% Tween 20 twice for five minutes, permeabilized with 0.5% Triton X-100 for ten minutes, and blocked with 5% FBS and 0.2% gelatin for 30 minutes. Then, the slices were stained with 5 μ g/mL anti-EGFR (clone A19002A) Alexa Fluor® 647 (red) at 4°C overnight. Nuclei were counterstained with DAPI (green). The image was captured with a 10X objective.

Recommended Usage:

Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 μL per million cells in 100 μL staining volume or 5 μL per 100 μL of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.

Brilliant Violet 750™ excites at 405 nm and emits at 750 nm. The bandpass filter 780/60 nm is recommended for detection, although filter optimization may be required depending on other fluorophores used. Be sure to verify that your cytometer configuration and software setup are appropriate for detecting this channel. Refer to your instrument manual or manufacturer for support. Brilliant Violet 750™ is a trademark of Sirigen Group Ltd.

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Application Notes:

Additional reported applications (for the relevant formats) include: immunoprecipitation, immunohistochemical staining of acetone-fixed frozen tissue sections⁴, and *in vitro* T cell costimulation⁵⁻⁸. This clone was tested in-house and does not work on formalin fixed paraffin-embedded (FFPE) tissue. The CD28.2 antibody co-stimulates T cell proliferation and cytokine production in the presence of suboptimal amounts of anti-CD3 antibody.

Application References:

- 1. Schlossman S, et al. Eds. 1995. Leucocyte Typing V. Oxford University Press. New York.
- 2. Nunes J, et al. 1993. Biochem. J. 293:835.
- 3. Calea-Lauri J, et al. 1999. J. Immunol. 163:62.
- 4. Tazi A, et al. 1999. J. Immunol. 163:3511. (IHC)
- 5. Marti F, et al. 2001. J. Immunol. 166:197. (Costim)
- 6. Jeong SH, et al. 2004. J. Virol. 78:6995. (Costim)
- 7. Rivollier A, et al. 2004. Blood 104:4029. (Costim)
- 8. Scharschmidt E, et al. 2004. Mol. Cell Biol. 24:3860. (Costim)
- 9. Sheng W, et al. 2007. Elsevier 580:6819. PubMed
- 10. Mitsuhashi M. 2007. Clin Chem.53:148. PubMed
- 11. Ye Z, et al. 2008. Infect. Immun. 76:2541. PubMed
- 12. Magatti M, et al. 2008. Stem Cells 26:182. (FA) PubMed
- 13. Yoshino N, et al. 2008. Exp. Anim. (Tokyo) 49:97. (FC)
- 14. Berg M, et al. 2008. J Leukoc Biol. 83:853. (IP) PubMed
- 15. Rout N, et al. 2010. PLoS One 5:e9787. (FC)
- 16. Leonard JA, et al. 2011. J. Virol. 85:6867. PubMed
- 17. Nomura T, et al. 2012. J. Virol. 86:6481. PubMed

Description:

CD28 is a 44 kD disulfide-linked homodimeric type I glycoprotein. It is a member of the immunoglobulin superfamily and is also known as T44 or Tp44. CD28 is expressed on most T lineage cells, NK cell subsets, and plasma cells. CD28 binds both CD80 and CD86 using a highly conserved motif MYPPY in the CDR3-like loop. CD28 is considered a major costimulatory molecule, inducing T lymphocyte activation and IL-2 synthesis, and preventing cell death. *In vitro* studies indicate that ligation of CD28 on T cells by CD80 and CD86 on antigen presenting cells provides a costimulatory signal required for T cell activation and proliferation.

Antigen References:

- 1. Schlossman S, et al. Eds. 1995. Leucocyte Typing V. Oxford University Press. New York.
- 2. June CH, et al. 1994. Immunol. Today 15:321.
- 3. Linskey PS, et al. 1993. Annu. Rev. Immunol. 11:191.