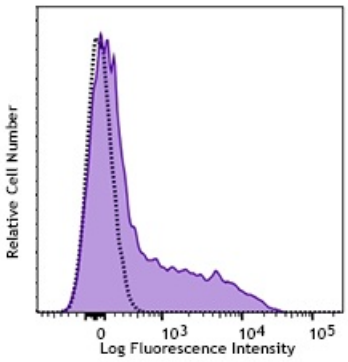
SONY

Product Data Sheet

Brilliant Violet 750™ anti-human CD137 (4-1BB)

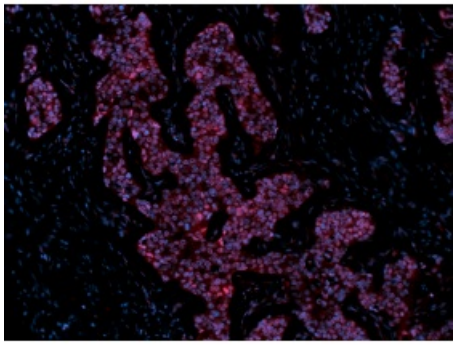
Catalog # / Size:	2149220 / 100 tests 2149215 / 25 tests
Clone:	4B4-1
Isotype:	Mouse IgG1, κ
Immunogen:	Ectodomain of recombinant human 4-1BB fusion protein
Reactivity:	Human, Non-human primate, Other
Preparation:	The antibody was purified by affinity chromatography and conjugated with Brilliant Violet 750™ under optimal conditions.
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)
Workshop Number:	VI C-7
Concentration:	Lot-specific



PHA-stimulated (three days) human peripheral blood lymphocytes were stained with CD137 (4-1BB) (clone 4B4-1) Brilliant Violet 750™ (filled histogram) or mouse IgG1, κ Brilliant Violet 750™ isotype control (open histogram).

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 µg/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.

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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.

Application Notes:

Additional reported applications (for the relevant formats) include: immunoprecipitation[1,4], inhibition of cytokine production[2,3], and ELISA. For most successful immunofluorescent staining results, it may be important to maximize signal over background by using a relatively bright fluorochrome-antibody conjugate (Cat. No. 2149020) or by using a high sensitivity, three-layer staining technique (e.g., including a biotinylated anti-mouse IgG second step (Cat. No. 2626515), followed by Streptavidin-PE (Cat. No. 2626020)).

Application References:

1. Garni-Wagner B, *et al.* 1996. *Cell. Immunol.* 169:91. (IP)
2. Salih HR, *et al.* 2000. *J. Immunol.* 165:2903. (FA)
3. Kienzle G, *et al.* 2000. *Int. Immunol.* 12:73. (FA)
4. Langstein J, *et al.* 1998. *J. Immunol.* 160:2488. (IP)

Description: CD137 is a 39 kD transmembrane protein also known as 4-1BB. It is expressed on activated T cells. CD137 is a type I membrane protein and a member of the tumor necrosis factor receptor superfamily. CD137 appears to be important for T cell proliferation and survival, and induces monocyte activation through its interaction with 4-1BB ligand.

Antigen References:

1. Gruss H, *et al.* 1995. *Blood* 85:3378.
2. Sica G, *et al.* 2000. *Adv. Exp. Med. Biol.* 465:355.
3. Alderson M, *et al.* 1994. *Eur. J. Immunol.* 24:2219.
4. Schwarz H, *et al.* 1996. *Blood* 87:2839.