Technical Data Sheet

Alexa Fluor® 647 Mouse anti-CD247 (pY142)

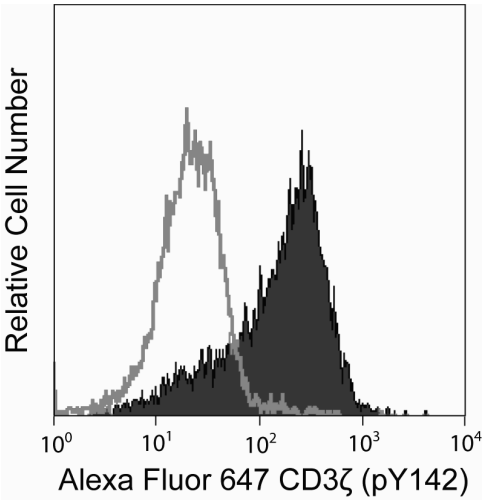
Product Information

Material Number:	**558489**
Alternate Name:	CD3ζ
Size:	50 tests
Vol. per Test:	20 µl
Clone:	K25-407.69
Immunogen:	Phosphorylated Human CD3ζ Peptide
Isotype:	Mouse IgG2a, κ
Reactivity:	Confirmed: Human Predicted: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The T cell receptor (TCR), expressed by thymocytes and T lymphocytes, is a multi-component cell-surface complex responsible for recognizing antigen in the context of MHC molecules. The antigen-specific binding component of the TCR, Ti, is a heterodimer of the variable Ig-like subunits α and β or γ and δ. Ti is non-covalently associated with an invariant set of molecules referred to as the CD3 polypeptides, γ, δ, ε, and ζ. The CD3 ζ polypeptide (CD3ζ) was named CD247 at the 7th Human Leukocyte Differentiation Antigens Workshop. CD3 appears early in thymocyte differentiation and remains expressed on all mature T lymphocytes. After antigen recognition by the TCR, CD3ζ is the primary intracellular signal transducing subunit. It contains three ITAMs (*I*mmunoreceptor *T*yrosine-based *A*ctivation *M*otifs), each of which contains a pair of tyrosine residues that are phosphorylated by Lck and Fyn and are required for signal propagation. The molecular weight of CD3ζ is 16 kDa, and it is also observed as 32-kDa homodimers or as heterodimers with the γ chain of Fc receptors. Upon phosphorylation, the CD3ζ monomer undergoes an apparent shift in electrophoretic mobility up to 21 kDa.

The K25-407.69 monoclonal antibody recognizes the phosphorylated tyrosine 142 (pY142) in the third ITAM domain of human CD3ζ (CD247).



Analysis of CD247 (CD3ζ) (pY142) in activated human T leukemia cells. *Jurkat cells (ATCC TIB-152) were either stimulated by cross-linking of CD3 and CD28 with NA/LE Mouse anti-Human CD3 mAb UCHT1 (Cat. No. 555329) and NA/LE Mouse anti-Human CD28 mAb CD28.2 (Cat. No. 555725) on ice for 15 minutes followed by Purified Goat anti-Mouse Ig (Cat. No. 553998) on ice for 15 minutes, and then allowed to undergo phosphorylation at 37°C for 2 minutes (shaded histogram) or unstimulated (open histogram). The cells were fixed (BD Phosflow™ Fix Buffer I, Cat. No. 557870) for 10-15 minutes at 37°C, permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, blocked with normal mouse immunoglobulin, and then stained with Alexa Fluor® 647 Mouse anti-CD247 (CD3ζ) (pY142). Flow cytometry was performed on a BD FACSCalibur™ 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 to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
557870	Fix Buffer I	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
555725	Purified NA/LE Mouse Anti-Human CD28	0.5 mg	CD28.2
553998	Polyclonal Goat Anti-Mouse Ig	0.5 mg	Polyclonal

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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. 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.
5. 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.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Alberola-Ila J, Takaki S, Kerner JD, Perlmutter RM. Differential signaling by lymphocyte antigen receptors. *Annu Rev Immunol.* 1997; 15:125-154. (Biology)

Ernst DN, Shih CC. CD3 complex. *J Biol Regul Homeost Agents.* 2000; 14(3):226-229. (Biology)

Kersh EN, Shaw AS, Allen PM. Fidelity of T cell activation through multistep T cell receptor ζ phosphorylation. *Science.* 1998; 281:572-575. (Biology)

Salomon AR, Ficarro SB, Brill LM, et al. Profiling of tyrosine phosphorylation pathways in human cells using mass spectrometry. *Proc Natl Acad Sci U S A.* 2003; 100(2):443-448. (Biology)

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