Technical Data Sheet

Alexa Fluor® 647 Mouse anti-WIP (pS488)

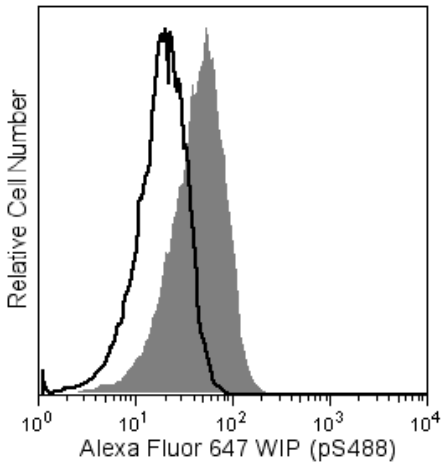
Product Information

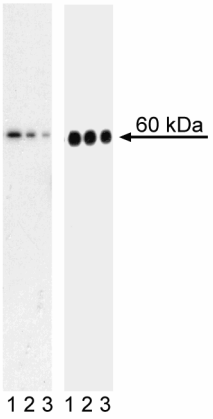
Material Number:	**558674**
Alternate Name:	PRPL-2 protein, WAIP, WASIP, WASPIP
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	K32-824
Immunogen:	Phosphorylated Human WIP Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Predicted Reactivity: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Wiskott-Adrich syndrome protein (*W*ASP)-*I*nteracting *P*rotein (WIP) is a member of the verprolin family of proteins that regulate cytoskeletal organization in a wide variety of cellular activities, including endocytosis, cellular adhesion and migration, mast cell degranulation, and lymphocyte activation. The 503-amino acid WIP protein contains binding sites for actin (globular and filamentous) and other proteins that are involved in the regulation of actin polymerization, such as WASP, N-WASP, profilin, cortactin, Hck, and NCK. As its functions imply, WIP is localized in actin-rich cell structures.

The K32-824 monoclonal antibody recognizes the phosphorylated serine 488 (pS488) of human WIP. The orthologous phosphorylation sites in mouse and rat WIP are S478 and S472, respectively.





Analysis of WIP (pS488) in human T leukemia cells. *LEFT: The shaded histogram displays Jurkat cells (ATCC TIB152) that were stimulated by cross-linking of CD3 and CD28 with NA/LE Mouse anti-Human CD3 mAb (Cat. No. 555329) and NA/LE Mouse anti-Human CD28 mAb (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 3 minutes. The open histogram shows unstimulated Jurkat cells. The cells were fixed using BD Cytofix™ buffer (Cat. No. 554655) for 10 minutes at 37°C, permeabilized usingBD 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-WIP (pS488). Flow cytometry was performed on a BD FACSArray™ bioanalyzer system. RIGHT: The specificity of mAb K32-824 was confirmed by western blot using unconjugated antibody on lysates from control (left panel) and CD3/CD28-cross-linked (right panel) Jurkat cells. WIP (pS488) is upregulated in the treated cells; its observed molecular weight is ~60 kDa, although the calculated molecular weight is 51 kDa.*

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.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 mL	(none)
558050	Perm Buffer III	125 mL	(none)
554656	Stain Buffer (FBS)	500 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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Antón IM, Jones GE. WIP: A multifunctional protein involved in actin cytoskeleton regulation. *Eur J Cell Biol.* 2006; 85:295-304. (Biology)
Aspenström P. The verprolin family of proteins: Regulators of cell morphogenesis and endocytosis. *FEBS Lett.* 2005; 579:5253-5259. (Biology)
Sechi AS, Wehland J. Interplay between TCR signalling and actin cytoskeleton dynamics. *Trends Immunol.* 2004; 25(5):257-265. (Biology)

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