Technical Data Sheet

Alexa Fluor® 488 Mouse anti–c-Cbl (pY700)

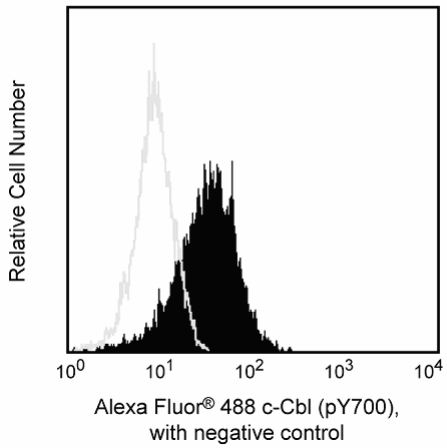
Product Information

Material Number:	**558101**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	47/c-Cbl
Immunogen:	Human c-Cbl (pY700) Peptide
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Cbl (***C***asitas ***B***-lineage ***l***ymphoma) was identified in the genome of a transforming retrovirus from a mouse pre-B lymphoma. The cellular gene product c-Cbl is one of numerous Cbl-related proteins found in vertebrate and invertebrate organisms. It is an 120-kDa adapter protein that contains multiple functional domains, including a RING finger motif, a tyrosine kinase-binding (TKB) domain, and a proline-rich region. The TKB domain directly interacts with specific auto-phosphorylation sites in activated protein-tyrosine kinases (PTK). Through the RING finger motif, c-Cbl recruits and activates an E2 ubiquitin-conjugating enzyme, thus targeting the activated PTK for protein degradation. The proline-rich region contains SH3 domain-binding and 14-3-3 protein-binding motifs. c-Cbl is also phosphorylated at tyrosines 700 (Y700), 731, and 774 by Syk- and Src-family kinases after the stimulation of some integrins and a wide variety of receptors for antigens, immunoglobulins, growth factors, cytokines, and hormones. In turn, the phosphorylated Y700 site interacts with the SH2 domains of CRK and Vav1. The c-Cbl adapter protein is expressed in the cytoplasm in all tissues, with especially high levels of expression in hematopoietic cells. Through its many functional sites, c-Cbl plays key roles in the positive and negative regulation of vital cell functions, including T Cell Receptor-mediated cellular immune responses.

The 47/c-Cbl monoclonal antibody recognizes the Y700-phosphorylated form of human c-Cbl. The orthologous phosphorylation site in mouse c-Cbl is Y698.



Analysis of c-Cbl (pY700) in activated human T leukemia cells. *Jurkat cells (ATCC TIB152) were serum starved overnight and then either stimulated with 5 mM hydrogen peroxide for 15 minutes (shaded histogram) or unstimulated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes, then permeabilized (BD™ PhosFlow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with Alexa Fluor® 488 anti-c-Cbl (pY700). Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

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. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.
7. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
8. 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.
9. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Miura-Shimura Y, Duan L, Rao NL, et al. Cbl-mediated ubiquitinylation and negative regulation of Vav. *J Biol Chem.* 2003; 278:38495-38504.(Biology)

Thien CBF, Langdon WY. CBL: Many adaptations to regulate protein tyrosine kinases. *Nat Rev Mol Cell Biol.* 2001; 2:294-307.(Biology)

Tsygankov AY, Teckchandani AM, Feshchenko EA, Swaminathan G. Beyond the RING: CBL proteins as multivalent adapters. *Oncogene.* 2001; 20:6382-6402. (Biology)