

Technical Data Sheet

PE Mouse anti-c-Cbl (pY774)

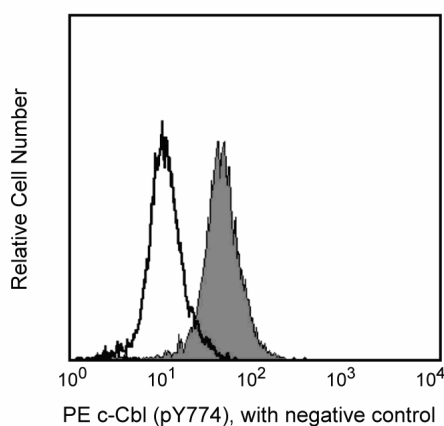
Product Information

Material Number:	558102
Size:	50 tests
Vol. per Test:	20 µl
Clone:	29/c-Cbl
Immunogen:	Phosphorylated Human c-Cbl Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Cbl (Casitas *B*-lineage lymphoma) 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, 731, and 774 (Y774) 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 Y774 site interacts with the SH2 domain of the CRK adapter protein. 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 29/c-Cbl monoclonal antibody recognizes the Y774-phosphorylated form of human c-Cbl.



Analysis of c-Cbl (pY774) 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™ Fixation 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 PE anti-c-Cbl (pY774). 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 with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)

Routinely Tested

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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- μ l experimental sample (a test).
2. Please refer to www.bdbiosciences.com/pharming/protocols for technical protocols.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.

References

Thien CBF, Langdon WY. CBL: Many adaptations to regulate protein tyrosine kinase. *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)

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