

Technical Data Sheet

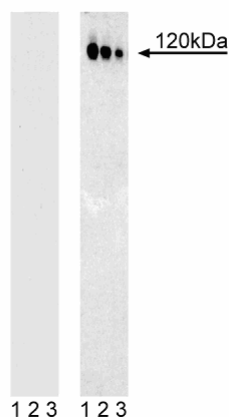
Purified Mouse Anti-c-Cbl (pY774)**Product Information**

Material Number:	558035
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	29/c-Cbl
Immunogen:	Phosphorylated Human c-Cbl Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

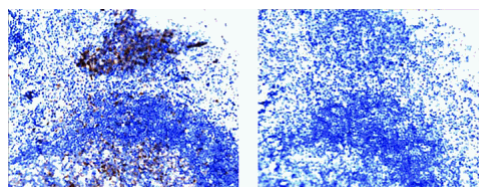
Description

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.



Western blot analysis of c-Cbl (pY774) in human T leukemia. Lysates from control (Cat. No. 611451, left panel) and Pervanadate-treated (Cat. No. 611755, right panel) Jurkat cells were probed with purified mouse anti-c-Cbl (pY774) monoclonal antibody at concentrations of 1.0, 0.5, and 0.25 $\mu\text{g/ml}$ (Lanes 1, 2, and 3, respectively). c-Cbl (pY774) is identified as a band of 120 kDa in the treated cells.



c-Cbl (pY774) staining on tonsil. Fresh human tonsil was incubated in 5 mM Pervanadate solution for 2 hours, then fixed in formalin and processed. Following antigen retrieval with BD Retrieval A buffer (Cat. no. 550524), the sections were either left untreated (left panel) or treated with a phosphatase to eliminate all phosphorylation (right panel). The tissue sections were stained with purified mouse anti-c-Cbl (pY774) with Hematoxylin counterstaining. Original magnification: 20X.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Store undiluted at 4°C.

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Application Notes

Application

Western blot	Routinely Tested
Immunohistochemistry-formalin (antigen retrieval required)	Tested During Development

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611451	Jurkat Cell Lysate	500 µg	(none)
611755	Jurkat + Pervanadate Lysate	500 µg	(none)
550524	Retrievagen A (pH 6.0)	1000 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/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.

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)