#3868 Store at -20°C

# LC3B (D11) XP® Rabbit mAb

Cell Signaling TECHNOLOGY®

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rev. 07/02/18

**For Research Use Only. Not For Use In Diagnostic Procedures.**

**Entrez-Gene ID** #81631
**UniProt ID** #Q9GZQ8

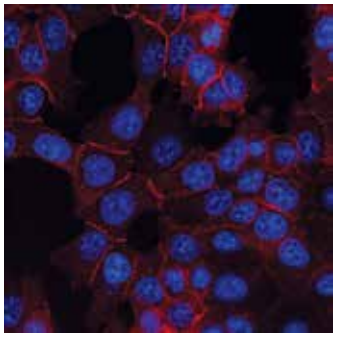
| Applications | Species Cross-Reactivity* | Molecular Wt. | Isotype |
|---|---|---|---|
| W, IP, IHC-P, IF-IC, F Endogenous | H, M, R, (Mk, B, Pg) | 14, 16 kDa | Rabbit IgG** |

**Background:** Autophagy is a catabolic process for the autophagosomic-lysosomal degradation of bulk cytoplasmic contents (1,2). Autophagy is generally activated by conditions of nutrient deprivation but has also been associated with a number of physiological processes including development, differentiation, neurodegenerative diseases, infection and cancer (3). Autophagy marker Light Chain 3 (LC3) was originally identified as a subunit of microtubule-associated proteins 1A and 1B (termed MAP1LC3) (4), and subsequently found to contain similarity to the yeast protein Apg8/Aut7/Cvt5 critical for autophagy (5). Three human LC3 isoforms (LC3A, LC3B, and LC3C) undergo post-translational modifications during autophagy (6-9). Cleavage of LC3 at the carboxy terminus immediately following synthesis yields the cytosolic LC3-I form. During autophagy, LC3-I is converted to LC3-II through lipidation by a ubiquitin-like system involving Atg7 and Atg3 that allows for LC3 to become associated with autophagic vesicles (6-10). The presence of LC3 in autophagosomes and the conversion of LC3 to the lower migrating form LC3-II have been used as indicators of autophagy (11).

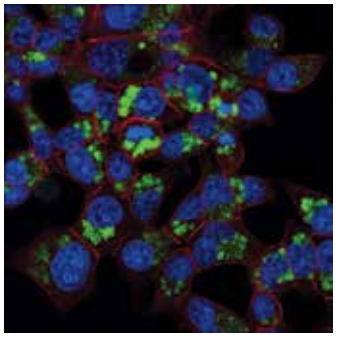
**Specificity/Sensitivity:** LC3B (D11) XP® Rabbit mAb detects endogenous levels of total LC3B protein. Cross-reactivity may occur with other LC3 isoforms. Stronger reactivity is observed with the type II form of LC3B. Weaker reactivity is observed with rodent LC3B.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of LC3B.

**Untreated**



**Chloroquine-treated**



◀ *Confocal immunofluorescent analysis of HCT-116 cells, untreated (upper) or choroquine-treated (50 µM, overnight; lower) using LC3B (D11) XP® Rabbit mAb (green) and β-Catenin (L54E2) Mouse mAb (Alexa Fluor® 555 Conjugate) #5612 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).*

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

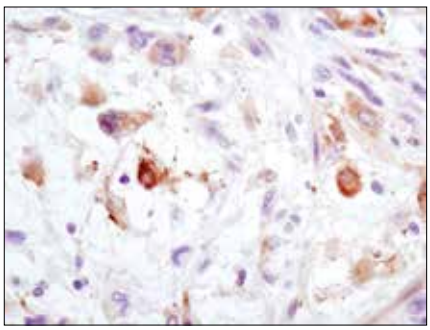
**Recommended Antibody Dilutions:**

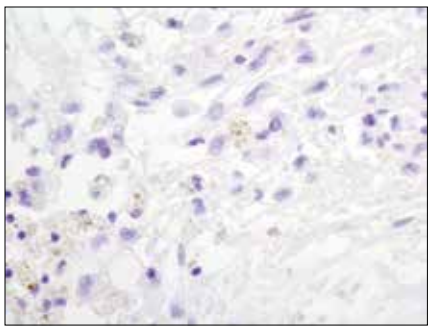
| | |
|---|---|
| Western blotting | 1:1000 |
| Immunoprecipitation | 1:50 |
| Immunohistochemistry (Paraffin) | 1:3200 |
| Unmasking buffer: | Citrate |
| Antibody diluent: | SignalStain® Antibody Diluent #8112 |
| Detection reagent: | SignalStain® Boost (HRP, Rabbit) #8114 |

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

| | |
|---|---|
| Immunofluorescence (IF-IC) | 1:200 |
| IF Protocol: | Methanol Fixation required |
| Flow Cytometry | 1:200 |

**For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com**





*Immunohistochemical analysis of paraffin-embedded human astrocytoma using LC3B (D11) XP® Rabbit mAb in the presence of control peptide (left) or antigen-specific peptide (right).*

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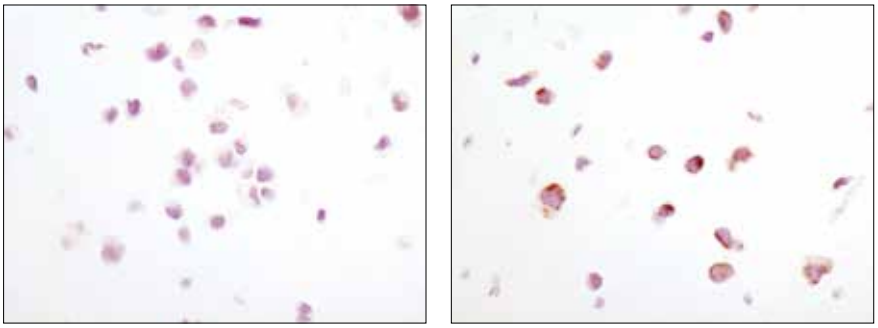
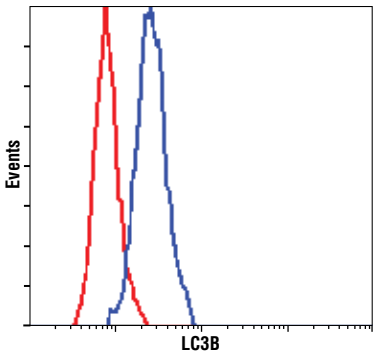
**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.**

**Applications Key:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

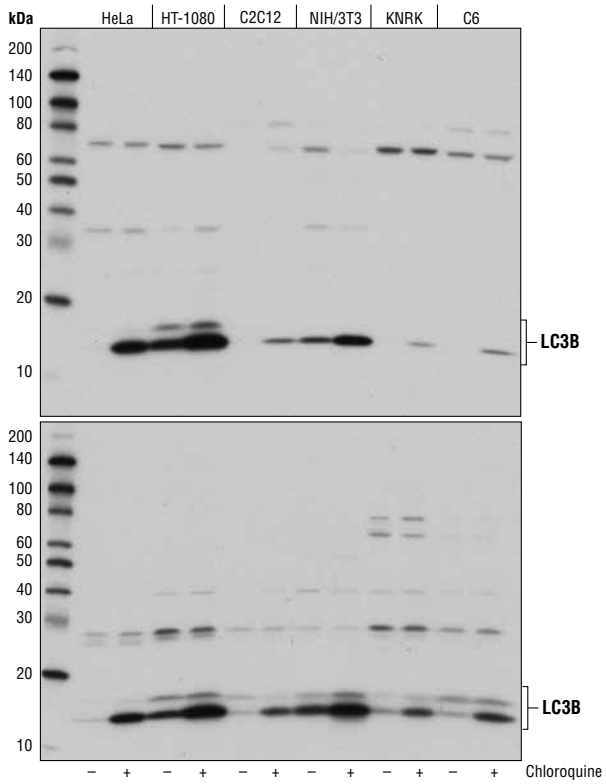
**Species Cross-Reactivity Key:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



*Immunohistochemical analysis of paraffin-embedded HeLa cell pellets, control (left) or chloroquine-treated (right), using LC3B (D11) XP® Rabbit mAb.*




*Flow cytometric analysis of HeLa cells using LC3B (D11) XP® Rabbit mAb (blue) compared to a nonspecific negative control antibody (red).*




*Western blot analysis of extracts from various cell lines, untreated (-) or treated overnight with chloroquine (50 µM) (+), using LC3B (D11) XP® Rabbit mAb (upper) or LC3B Antibody #2775 (lower).*

**Background References:**


(1) Reggiori, F. and Klionsky, D.J. (2002) *Eukaryot. Cell* 1, 11–21.

(2) Codogno, P. and Meijer, A.J. (2005) *Cell Death Differ.* 12 Suppl 2, 1509–1518.

(3) Levine, B. and Yuan, J. (2005) *J. Clin. Invest.* 115, 2679–2688.

(4) Mann, S.S. and Hammarback, J.A. (1994) *J. Biol. Chem.* 269, 11492–11497.

(5) Lang, T. et al. (1998) *EMBO J.* 17, 3597–3607.

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(10) Ichimura, Y. et al. (2000) *Nature* 408, 488–492.

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