#12741 Store at -20°C

LC3A/B (D3U4C) XP® Rabbit mAb

☐ Small 100 µl
(10 western blots)

☐ Petite 40 µl
(4 western blots)

Cell Signaling TECHNOLOGY®

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rev. 07/22/14

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

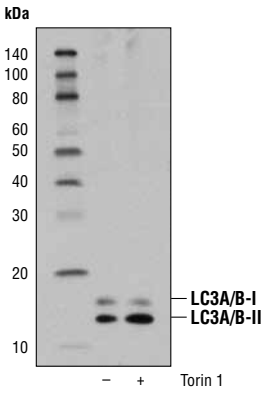
Entrez-Gene ID #84557, 81631
Swiss-Prot Acc. #Q9H492, Q9GZQ8

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

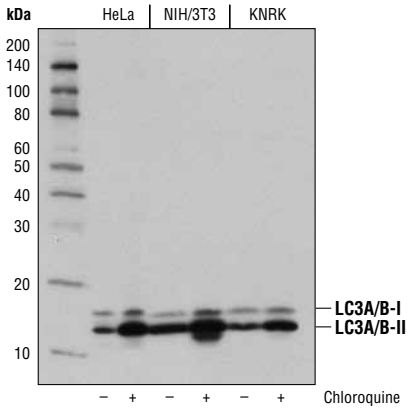
Background: Autophagy is a catabolic process for the autophagosomic-lysosomal degradation of bulk cytoplasmic contents (1,2). Autophagy is generally activated by conditions of nutrient deprivation, but it has also been associated with a number of physiological processes including development, differentiation, 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: LC3A/B (D3U4C) XP® Rabbit mAb recognizes endogenous levels of total LC3A and LC3B proteins.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Leu44 of human LC3B protein (conserved in LC3A).



Western blot analysis of extracts from RD cells, untreated (-) or Torin 1-treated (250 nM, 4 hr); using LC3A/B (D3U4C) XP® Rabbit mAb.



Western blot analysis of extracts from HeLa, NIH/3T3, and KNRK cells, untreated (-) or chloroquine-treated (50 µM, overnight); using LC3A/B (D3U4C) XP® Rabbit mAb.

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
Immunohistochemistry (Paraffin)	1:1000†
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)	1:100
IF Protocol:	Methanol Fixation required
Flow Cytometry	1:100

For product specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended complementary products.

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.

(6) Kabeya, Y. et al. (2000) *EMBO J.* 19, 5720-5728.

(7) He, H. et al. (2003) *J. Biol. Chem.* 278, 29278-29287.

(8) Tanida, I. et al. (2004) *J. Biol. Chem.* 279, 47704-47710.

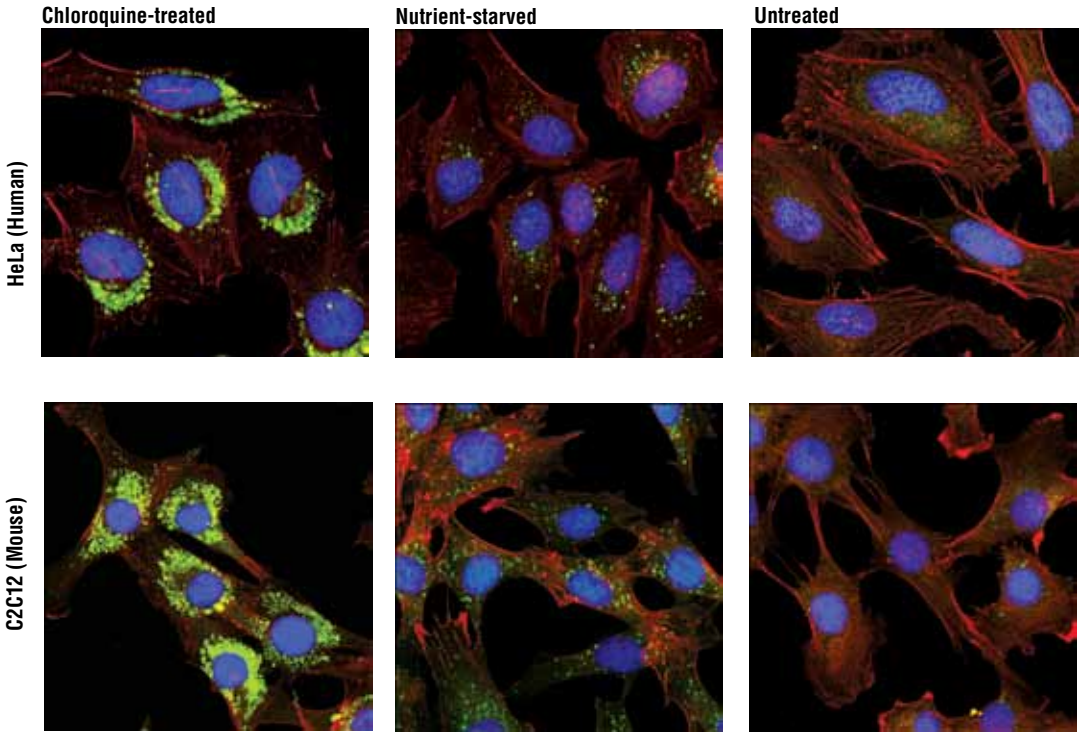
(9) Wu, J. et al. (2006) *Biochem. Biophys. Res. Commun.* 339, 437-442.

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

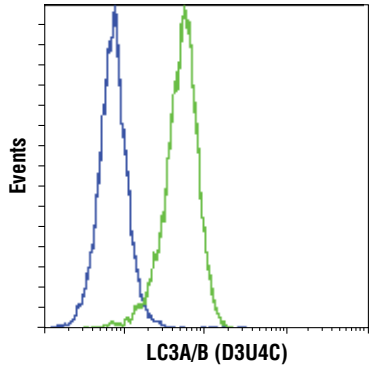
(11) Kabeya, Y. et al. (2004) *J. Cell Sci.* 117, 2805-2812.

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.

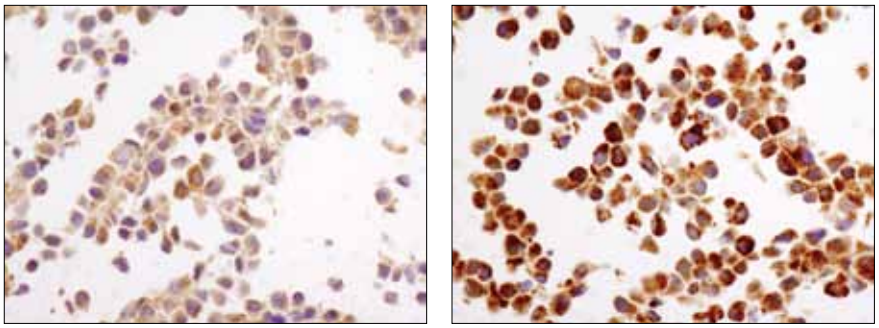
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **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.



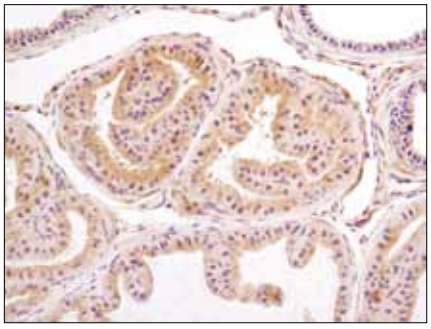
Confocal immunofluorescent analysis of HeLa (upper) and C2C12 (lower) cells, chloroquine-treated (50 µM, overnight; left), nutrient-starved with EBSS (3 hr, middle) or untreated (right) using LC3A/B (D3U4C) XP® Rabbit mAb (green) and β-Actin (13E5) Rabbit mAb (Alexa Fluor® 555 Conjugate) #8046 (red). Blue pseudocolor= DRAQ5® #4084 (fluorescent DNA dye).



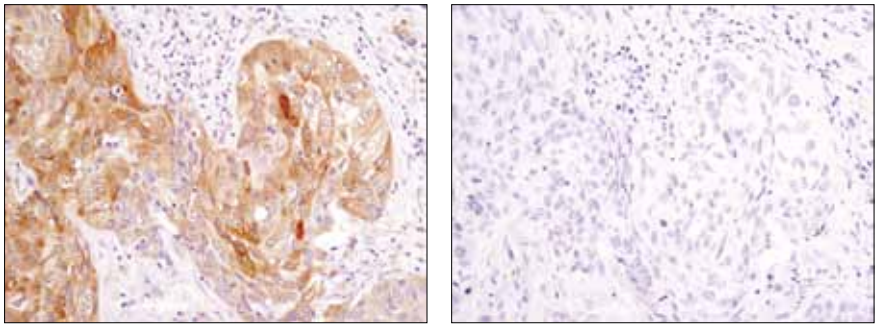
Flow cytometric analysis of HeLa cells, untreated (blue) or treated with chloroquine (50 µM, 16 hr) (green), using LC3A/B (D3U4C) Rabbit mAb. Anti-rabbit IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 647 Conjugate) #4414 was used as a secondary antibody.



Immunohistochemical analysis of paraffin-embedded NIH/3T3 cell pellets, control (left) or chloroquine-treated (right), using LC3A/B (D3U4C) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded mouse prostate using LC3A/B (D3U4C) XP® Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human lung carcinoma using LC3A/B (D3U4C) XP® Rabbit mAb in the presence of control peptide (left) or antigen-specific peptide (right).