

LC3A (D50G8) XP® Rabbit mAb

✓ 100 µl
 (10 western blots)



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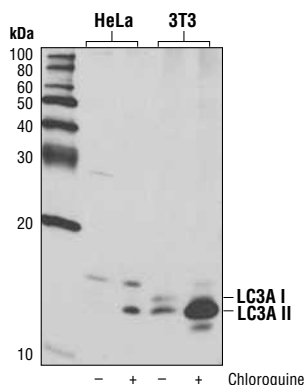
rev. 12/30/11

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

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

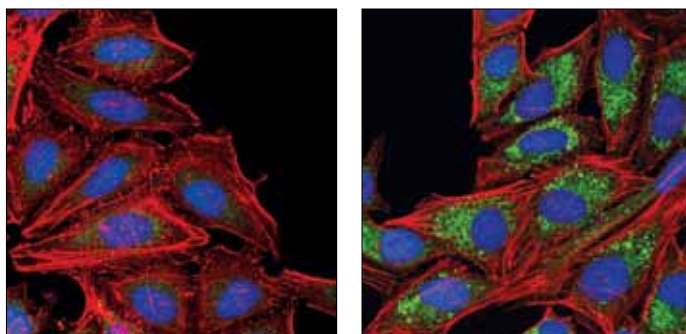
Background: Autophagy is a catabolic process for the autophagosomal-lysosomal degradation of bulk cytoplasmic contents (1,2). Autophagy is generally activated by conditions of nutrient deprivation but has also been associated with a number of physiological processes including development, differentiation, 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: LC3A (D50G8) XP® Rabbit mAb detects endogenous levels of total LC3A protein. This antibody may also react with LC3B.



Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated or chloroquine-treated (50 µM, overnight), using LC3A (D50G8) XP® Rabbit mAb.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human LC3A.



Confocal immunofluorescent analysis of HeLa cells, untreated (left) or chloroquine-treated (right), using LC3A (D50G8) XP® Rabbit mAb (green). Actin filaments were labeled using DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

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.

Entrez-Gene ID #84557
 Swiss-Prot Acc. #Q9H492

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. 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 | 1:100 |
| Immunohistochemistry (Paraffin) | 1:6400† |
| 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) | 1:400 |
| IF Protocol: | Methanol Permeabilization required |
| Flow Cytometry | 1:100 |

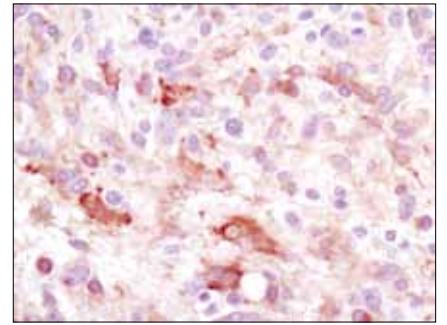
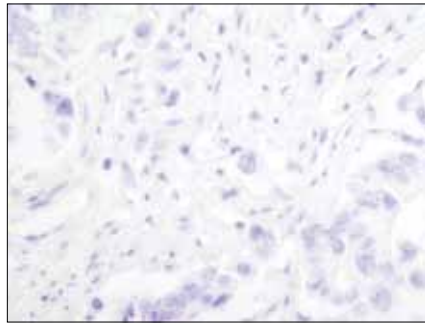
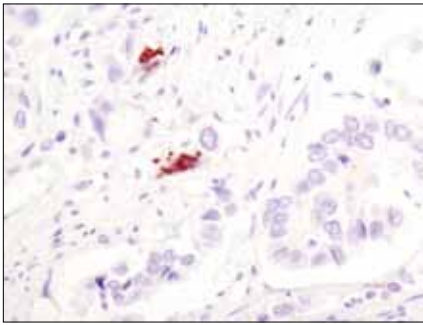
For application specific protocols please see the web page for this product at www.cellsignaling.com.

Please visit www.cellsignaling.com for a complete listing of recommended companion products.

Background References:

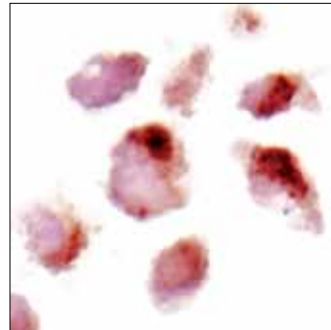
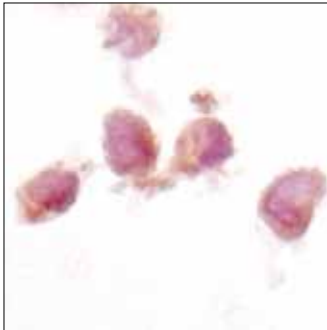
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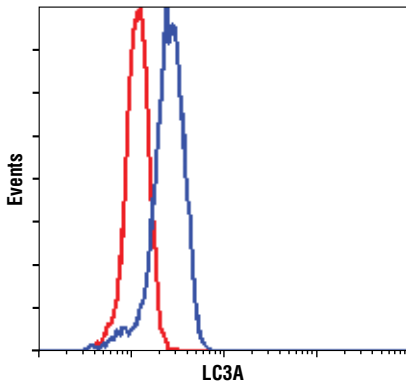


Immunohistochemical analysis of paraffin-embedded human colon carcinoma using LC3A (D50G8) XP[®] Rabbit mAb (left) or Rabbit (DA1E) mAb IgG XP[®] Isotype Control #3900 (right).

Immunohistochemical analysis of paraffin-embedded human glioblastoma multiforme using LC3A (D50G8) XP[®] Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded HeLa cell pellets, control (left) or chloroquine-treated (right), using LC3A (D50G8) XP[®] Rabbit mAb.



Flow cytometric analysis of chloroquine-treated HeLa cells, using LC3A (D50G8) XP[®] Rabbit mAb (blue) compared to Rabbit (DA1E) mAb IgG XP[®] Isotype Control #3900 (red).