

# LC3 Control Cell Extracts



✓ 100 µl  
 (10 western blots)

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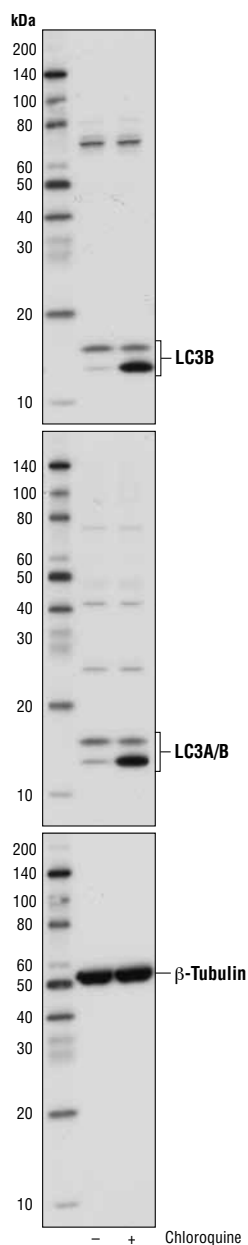
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**Description:** Total cell extracts from HeLa cells were untreated or treated with 50 µM chloroquine overnight. This lysate pair is produced as a control for western blotting of LC3A and LC3B. LC3C cannot be detected in these lysates. Boil for 2 minutes in the original tube, then load 10 µl per mini-gel lane.

**Background:** Autophagy is a catabolic process for the autophagosome-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).

## Background References:

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- (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.
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- (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.



Western blot analysis of LC3 Control Cell Extracts from HeLa cells, untreated (-) or chloroquine-treated (50 µM, overnight; +), using LC3B (D11) XP® Rabbit mAb #3868 (upper), LC3A/B Antibody #4108 (middle), or β-Tubulin (9F3) Rabbit mAb #2128 (lower).

**Storage:** Supplied in SDS Sample Buffer: 62.5 mM Tris- HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red. Store at -20°C, or at -80°C for long-term storage.

**For product specific protocols and a complete listing of recommended companion products please see the product web page at [www.cellsignaling.com](http://www.cellsignaling.com)**

**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.