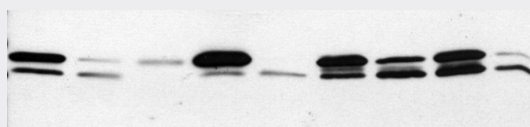


Autophagic Marker LC3

microtubule-associated protein1 light chain 3

Immunoblot Analysis of LC-3



LC3 is routinely detected in different cell lines (1: HeLa; 2: HepG2; 3: HEK 293; 4: SH-SY5Y; 5: MDCK; 6: PC12; 7: CMT; 8: Neuro 2A; 9: NIH 3T3). Whole cell extracts of untreated cells containing approx. 20.000 cells per lane were applied on SDS-PAGE and transferred to PVDF membrane. The immunoblot was probed with Mab LC3-5F10 at 0.5µg/ml for 1h at 15-22°C followed by HRP-conjugated secondary antibody (developed with ECL, exposure time: 30 sec).

BACKGROUND

Autophagy is a process responsible for the bulk degradation of intracellular material that is evolutionarily conserved between all eukaryotes. In autophagy, cytoplasmic components are engulfed by double-membrane-bound structures (autophagosomes) and delivered to lysosomes/vacuoles for degradation. Recent reports indicate that autophagy plays a crucial role in many different pathologies. Indeed, both activation and inactivation of autophagy could benefit cancer cells. If cells cannot activate autophagy, protein synthesis predominates over protein degradation and tumor growth is stimulated. By contrast, autophagy could be activated in more advanced stages of cancer to guarantee survival of cells in low-vascularized tumors. Furthermore, alterations in the autophagic-lysosomal compartment have been linked to neuronal death in many neurodegenerative disorders as well as in transmissible neuronal pathologies (prion diseases). Activation of autophagy could also be an effective way of eliminating infectious agents that access the cytosol. Finally, autophagic vacuoles have been reported frequently in cardiomyopathies or muscle cells exposed to different experimental settings.

Although the importance of autophagy is undisputed, analysis of autophagy has been hampered by lack of good markers. Here we describe new monoclonal antibodies against MAP-LC3 (LC3). MAP-LC3 is a major constituent of the autophagosome, a double membraned structure that sequesters the target organelle/protein and then fuses with endo/lysosomes where the contents - and LC3 - are degraded.

SUMMARY

To date, LC3, a mammalian homolog of yeast Atg8, is the only reliable marker of autophagosomes.

Tracking the conversion of LC3-I to LC3-II is indicative of autophagic activity.

As half-life of LC3-II is short because autophagosomes are transient structures, LC3-II dots represent the autophagic activity at the moment in time.

Immunoblotting of LC3 usually reveals two bands: LC3-I (18 kDa) and LC3-II (16 kDa). The amount of LC3-II correlates well with the number of autophagosomes. This characteristic conversion of LC3 can be used to monitor autophagic activity.

It will be interesting to see how the analysis of endogenous LC3 conversion and cellular distribution using this novel monoclonal antibody may contribute to better insights of autophagy under physiological conditions and its relationship to apoptotic cell death.

During autophagy, the cytoplasmic form (LC3 I) is processed and recruited to the autophagosomes, where LC3 II is generated by site specific proteolysis and lipidation near to the C-terminus. The hallmark of autophagic activation is thus the formation of cellular autophagosome punctae containing LC3 II, while autophagic activity is measured biochemically as the amount of LC3 II that accumulates in the absence or presence of lysosomal activity.

Mab LC3-5F10 can be used to analyse both LC3 forms and is suitable for detecting endogenous LC3 protein by immunoblot and immunocytochemical applications, thereby being a valuable tool to study autophagy in cells.



Monoclonal Antibody against LC3

(microtubule-associated protein1 light chain 3)

Order No.: 0231-100/LC3-5F10
Clone Name: 5F10
Isotype: IgG
Host: Mouse
Size: 100 µg
Modifications: Please inquire

Specificity: LC3-I (18 kDa); LC3-II (16 kDa)
Immunogen: Synthetic peptide derived from the N-terminus of LC3, conjugated to KLH
Species Reactivity: Human, mouse, rat, dog, hamster

Positive Control: Neuro 2A, untreated
Applications: WB, ELISA, ICC

Introductory Price*: **EURO 316,-**

*valid until end of 2006

Related Products:

Order No.	Specificity	Size:	Price:
0060-100/bA4(40)-5C3	Amyloid bA4 (1-40), C-Terminus	100 µg	EURO 265,-
0061-100/bA4(42)-8G7	Amyloid bA4 (1-42), C-Terminus	100 µg	EURO 265,-
0062-100/bA4(40/42)	Amyloid bA4 (1-40/42), C-Terminus	100 µg	EURO 265,-
0095-100/bA4(43)-6G12	Amyloid bA4 (1-43), C-Terminus	100 µg	EURO 265,-
0064-100/bA4N-19H5	Amyloid bA4, N-Terminus	100 µg	EURO 265,-
0084-100/bA4N-19H11	Amyloid bA4, N-Terminus	100 µg	EURO 265,-
0197-100/bA4N-11H3	Amyloid bA4, N-Terminus	100 µg	EURO 265,-
0049-100/PKB-11A11	PKB/Akt (dephospho-Ser 473)	100 µg	EURO 316,-
0108-100/PKB-11E6	PKB/Akt (phospho-Ser 473)	100 µg	EURO 316,-
0119-100/PKB-5C10	PKB/Akt (C-Terminus)	100 µg	EURO 316,-
0144-100/PKBa-5G12	PKBapha/Akt1	100 µg	EURO 316,-
0179-100/PKBb-8B7	PKBbeta/Akt2	100 µg	EURO 316,-

Contact & Orders:

nanoTools Antikoerpertechnik GmbH & Co. KG
 Tscheulinstr. 21 . D-79331 Teningen . Germany
 Tel: +49-(0)7641-455 670
 fax: +49-(0)7641-455 671
 email: info@nanoTools.de
www.nanotools.de

