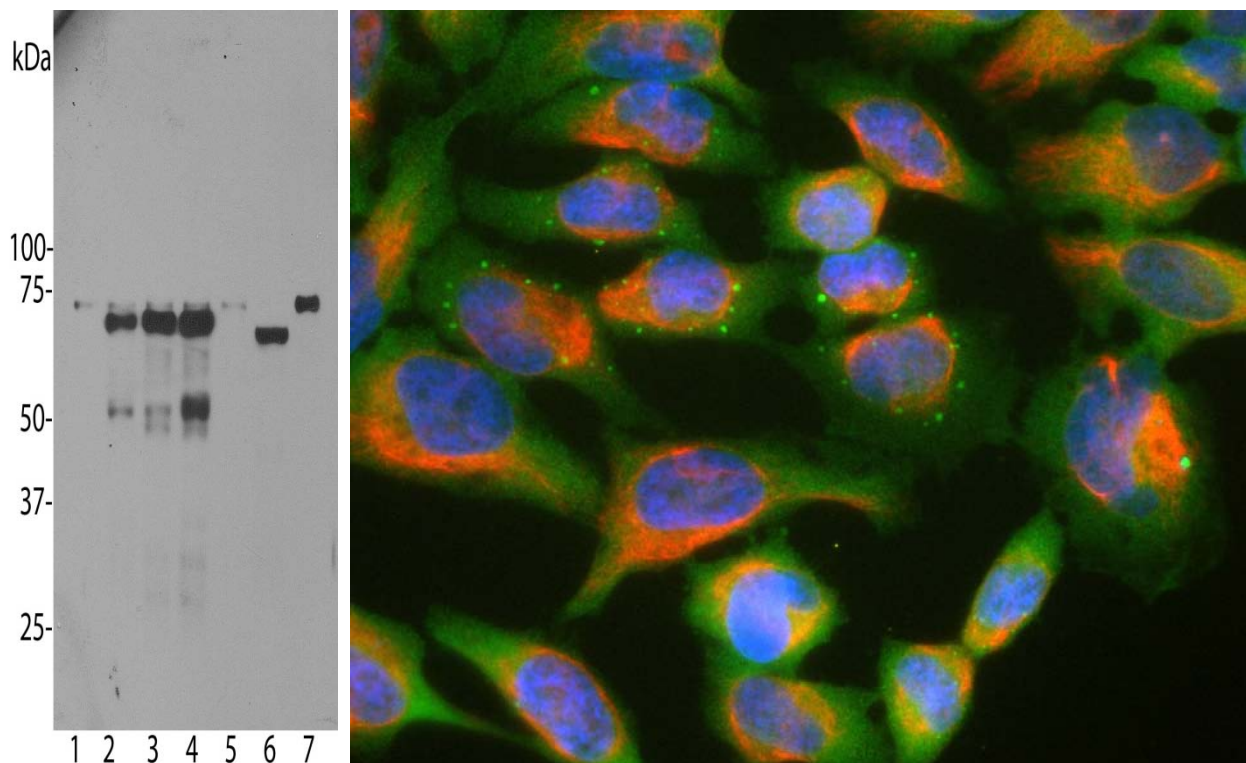


**Catalogue# MCA-6H9: Mouse Monoclonal Antibody to Ubiquilin 2**

**The Immunogen:** Ubiquilin 2, also known as PLIC2 and Chap1, is a member of the ubiquilin protein family, which regulate the degradation of cellular proteins through proteasome or autophagy-like pathways (1, 2, 3). Humans have four ubiquilin genes, each encoding a separate protein referred to as Ubiquilin 1, 2, 3 and 4. All ubiquilins contain an N-terminal ubiquitin-like (UBL) domain and a C-terminal ubiquitin-associated (UBA) domain, while the central part of the molecules are highly variable. The UBL domains bind subunits of the proteasome, and the UBA domains binds to polyubiquitin chains that are typically conjugated onto proteins marked for proteosomal degradation (1).

Ubiquilin 2 has a unique region close to the C terminus containing 12 PXX tandem collagen like repeats, where P is proline and X is most cases valine, glycine, isoleucine or threonine. Teepu Siddique and his collaborators have identified mutations in the ubiquilin 2 gene leading to protein point mutations which were important contributors to several forms of [amyotrophic lateral sclerosis \(ALS\)](#) and [Frontotemporal lobar degeneration \(FTLD\)](#). Interestingly, these mutations involved alterations in proline residues in the PXX repeat region (P497H, P497S, P506T, P509S and P525S, ref. 4). Recently, the Lee and Trojanowski group investigated C9orf72 hexanucleotide expansion and ubiquilin 2 pathology in patients with ALS and FTLD by genetic analysis and immunohistochemistry and found distinct ubiquilin 2 pathology in ALS and FTLD-TDP with C9orf72 expansion (5). C9orf72 hexonucleotide expansion is the most common cause to date of familial ALS and FTLD (6, 7).

Ubiquilin 2 protein is of different molecular size in mouse and human, 638 and 624 amino acids respectively. As a result the mouse protein, endogenously expressed in rodent 3T3 cells, runs on SDS-PAGE and western blots slightly slower than the human protein. Our antibody MCA-6H9 was raised against human ubiquilin 2 expressed in and purified from *E. coli*. The [HGNC](#) name for this protein is [UBQLN2](#).



**Left:** Western blot analysis of untransfected primary mouse neuron and glia cell cultures (lane 1), the same cells transduced with human ubiquilin 2 wild type (lane 2), with ubiquilin 2 P506T mutant (lane 3), with ubiquilin 2 P497S mutant (lane 4) and with enhanced GFP control (lane 5), all probed with MCA-6H9. Also seen is MCA-6H9 staining in HeLa cells (lane 6) and 3T3 cells (lane 7). In primary mouse neuron and glia cell culture, endogenous ubiquilin 2 appears as a weak band at 68 kDa in all transduced and non-transduced cells, indicating low endogenous expression of mouse ubiquilin 2. Strong bands are seen in the cells transduced with human wild type or mutant ubiquilin 2. Small proteins run at 50 kDa in these cells are the fragments of ubiquilin 2. Note, ubiquilin 2 run at ~66 kDa in human HeLa cells and 68 kDa in rodent 3T3 cells. **Right:** HeLa cell cultures stained with MCA-6H9 (green) and EnCor's chicken polyclonal antibody to vimentin: [CPCA-Vim](#) (red). In most individual cells ubiquilin 2 is present diffusely in the cytoplasm of cells, though some cells show enrichment of the protein in spherical autophagosome-like structure.

**Antibody characteristics:** MCA-6H9 is a mouse IgG1 class antibody and is known to react with ubiquilin 2 from human, cow, pig, mouse, rat and other mammals.

**Suggestions for use:** The antibody is affinity purified from tissue culture supernatant and is at concentration of 1 mg/mL in phosphate buffered saline. The antibody solution can be used at dilutions of at least 1:1,000 in immunofluorescence experiments. In western blotting using chemiluminescence it can be used at dilutions of 1:1,000-1:2,000 or lower. Antibody preparation contains 10 mM sodium azide preservative (<http://www.encorbio.com/MSDS/azide.htm> for Material Safety Data Sheet). Avoid repeated freezing and thawing, store at 4°C or -20°C.

#### References:

1. Kleijnen MF, Shih AH, Zhou P, Kumar S, Soccio R E, Kedersha N L, Gill G, Howley PM. The hPLIC proteins may provide a link between the ubiquitination machinery and the proteasome. [Molec. Cell 6: 409-419 \(2000\).](#)
2. N'Diaye EN, Kajihara KK, Hsieh I, Morisaki H, Debnath J, Brown EJ. PLIC proteins or ubiquilins regulate autophagy-dependent cell survival during nutrient starvation. [EMBO Rep. 10:173-9 \(2009\).](#)
3. Rothenberg C, Srinivasan D, Mah L, Kaushik S, Peterhoff CM, Ugolino J, Fang S, Cuervo AM, Nixon RA, Monteiro MJ. Ubiquilin functions in autophagy and is degraded by chaperone-mediated autophagy. [Hum Mol Genet. Aug 15; 19 \(16\): 3219-32. Epub Jun 7 \(2010\).](#)
4. Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, Yang Y, Fecto F, Shi Y, Zhai H, Jiang H, Hirano M, Rampersaud E, Jansen GH, Donkervoort S, Bigio EH, Brooks BR, AjroudK, Sufit RL, Haines JL, Mugnaini E, Pericak-Vance MA, Siddique T. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. [Nature Aug 21; 477\(7363\):211-5 \(2011\).](#)
5. Brettschneider J, Van Deerlin VM, Robinson JL, Kwong L, Lee EB, Ali YO, Safren N, Monteiro MJ, Toledo JB, Elman L, McCluskey L, Irwin DJ, Grossman M, Molina-Porcel L, Lee VM, Trojanowski JQ. Pattern of ubiquilin pathology in ALS and FTLD indicates presence of c9orf72 hexanucleotide expansion. [Acta Neuropathol. Jun; 123 \(6\):825-39 \(2012\).](#)
6. Renton AE, Majounie E, Waite AA, et al. Hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. [Neuron. Oct 20; 72\(2\):257-68 \(2011\).](#)
7. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. [Neuron. Oct 20; 72\(2\):245-56 \(2011\).](#)

**Limitations:** This product is for research use only and is not approved for use in humans or in clinical diagnosis.