

Ordering Information
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HGNC Name: UBB, UBC, UBA52, RPS27A
UniProt: P0CG48, P0CG47, P62987, P62979
RRID: AB_2572391
Immunogen: Purified bovine erythrocyte ubiquitin coupled to KLH with glutaraldehyde
Format: Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM Na₂S₂O₃
Storage: Store at 4°C for short term, for longer term at -20°C
Recommended dilutions:
 WB: 1:1,000-1:2,000. ICC/IF: 1:2,000. IHC: 1:2,000.

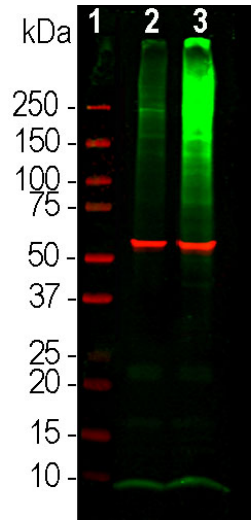
References:

- Goldstein G, et al. Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. *PNAS* 72:11-5 (1975).
- Wilkinson K. The discovery of ubiquitin-dependent proteolysis. *PNAS* 102:15280-2 (2005).
- Perry G, et al. Ubiquitin is detected in neurofibrillary tangles and senile plaque neurites of Alzheimer disease brains. *PNAS* 84:3033-6 (1987).
- Kuzuhara S, et al. Lewy bodies are ubiquitinated. A light and electron microscopic immunocytochemical study. *Acta Neuropathol.* 75:345-53 (1988).
- Murayama S, et al. Immunocytochemical and ultrastructural studies of Pick's disease. *Ann. Neurol.* 27:394-405 (1990).
- Shaw G, Chau V. Ubiquitin and microtubule-associated protein tau immunoreactivity each define distinct structures with differing distributions and solubility properties in Alzheimer brain. *PNAS* 85:2854-8 (1988).

This antibody has been widely used for about 30 years, though mostly sold through our OEM partners. Some of the papers which make use of the antibody supplied by EnCor can be found by searching Google Scholar for "MCA-Ubi AND antibody" or, if you are reading this online, go [here](#).

for a very recent example of the use of MCA-Ubi-1 on western blots to visualize ubiquitin conjugates go to figure 4e in Gladcova C, et al. Mechanism of parkin activation by PINK1. *Nature* 559:410-414 (2018).

| Applications | Host | Isotype | Molecular Wt. | Species Cross-Reactivity |
|-----------------|-------|---------------------|---------------|--|
| WB, IF/ICC, IHC | Mouse | IgG1 heavy, κ light | 8.5kDa | Hu, Mo, Rt, Ms, Ho, Co, Pi, Ch, Dr, Dm, Ce |

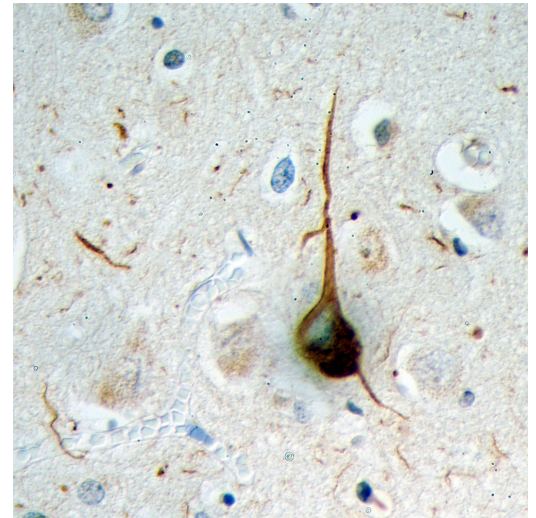


Western blot analysis of HEK293 cell lysates using mouse mAb to ubiquitin, MCA-Ubi-1, dilution 1:1,000 in green. [1] protein standard (red), [2] cells maintained in normal medium, [3] cells treated with 10µM of proteasome inhibitor lactacystin (Lc) for 16hrs. Lysed cells were electrophoresed on 4-20% SDS-PAGE, and transferred to PVDF membranes. The smear detected above the 200kDa standard represent accumulation of ubiquitinated proteins in proteasome inhibitor-Lc treated cells. The prominent band at 8kDa corresponds to monoubiquitin. The same blot was probed with rabbit pAb to HSP60, *RPCA-HSP60*, dilution 1:5,000 in red, used as a loading control.

Background:

Ubiquitin is a globular 76 amino acid protein of about 8.5kDa molecular weight which was discovered by biochemical isolation from bovine thymus tissues (1). The protein was found to be highly conserved in amino acid sequence and was detectable in apparently every cell and tissue type, and, being apparently ubiquitously expressed, became known as ubiquitin. Subsequent work showed that ubiquitin has an important role in the targeting of proteins for proteolytic degradation, but has other important functions (2). Proteins to be degraded are covalently coupled to the C-terminus of ubiquitin as a result of the sequential activity of three families of enzymes, the ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s). While humans express only two E1 enzymes, there are 35 E2 enzymes and hundreds of E3s. The ubiquitinated protein complex may then be degraded in the proteasome. Ubiquitin becomes covalently bonded to many types of pathological inclusions such as the neurofibrillary tangles of Alzheimer's disease (3), the Lewy bodies of Parkinson's disease (4), the Pick bodies of Pick's disease (5) and many others. Ubiquitin can normally be removed from proteins to which it is bound and reused due to the activity of a large family of deubiquitinating enzymes.

This antibody, MCA-Ubi-1, was made in the University of Florida in 1987, and has been continuously marketed since 1989 being sold by EnCor and numerous other vendors. The immunogen was purified bovine blood derived ubiquitin coupled to keyhole limpet hemocyanin with glutaraldehyde (6). The MCA-Ubi-1 is relatively insensitive to formalin fixation and so can be used on paraffin embedded fixed histological sections of human brain for studies of Alzheimer's and other neurodegenerative diseases. Epitope mapping of MCA-Ubi-1 was performed by generating a series of staggered 20 amino acid peptides which covered the human sequence with 5 amino acid overlap between neighboring peptides. Only the peptide, IQDKEGIPPDQQRLLIFAGKQ, amino acids 30-49, inhibited binding of MCA-Ubi-1 to purified bovine ubiquitin, see [here](#). Since the previous and next peptides had no apparent inhibitory effect on antibody binding, the central 10 amino acid segment, GIPPDQQRLL, is likely the most significant component of the MCA-Ubi-1 epitope. This peptide is totally conserved in animals, plants and fungi so MCA-Ubi-1 is applicable to studies of a wide variety of species from human to yeast. MCA-Ubi-1 also works on western blots and can be used to study ubiquitinated proteins which typically accumulate as a high molecular weight smear on western blots if the proteasome is inhibited. We also supply a rabbit polyclonal antibody to ubiquitin, *RPCA-Ubi*.



Formalin fixed paraffin embedded section of cerebral cortex of an Alzheimer patient processed with MCA-Ubi-1 using HRP/DAB, giving a brown signal. Also stained with haematoxylin in blue. A typical flame shaped tangle is seen in a pyramidal neuron in the center and is surrounded by some dystrophic neurites, also strongly ubiquitin positive. Both are commonly seen in cortical and hippocampal Alzheimer brain sections and are typical for this disease, but are rare or absent in healthy brain.

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Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.

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