

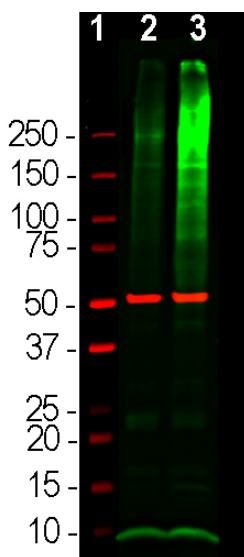
Ordering Information
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HGNC Name: UBB, UBC, UBA52, RPS27A
UniProt: P0CG48, P0CG47, P62987, P62979
RRID: AB_2253901
Immunogen: Purified ubiquitin conjugated with glutaraldehyde to KLH
Format: Supplied as an aliquot of serum plus 5mM NaH₂PO₄
Storage: Storage for short term at 4°C recommended, for longer term at -20°C, minimize freeze/thaw cycles
Recommended dilutions:
 WB: 1:5,000-1:10,000. IF/ICC and IHC: 1:500-1:1,000.

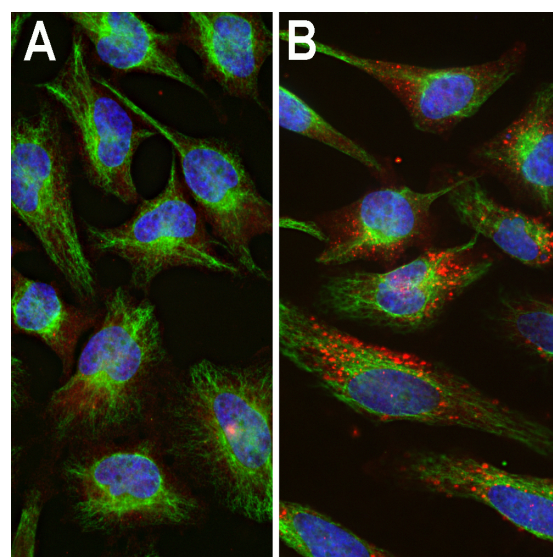
References:

- 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).

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, ICC/IF, IHC	Rabbit		8.5kDa	Hu, Rt, Ms



Western blot analysis of HEK293 cell lysates using rabbit pAb to ubiquitin, RPCA-Ubi, dilution 1:5,000 in green and mouse mAb to β -Tubulin, MCA-1B12, dilution 1:10,000, in red, used as a loading control. [1] protein standard (red), [2] cells maintained in normal medium, [3] cells treated with proteasome inhibitor lactacystin (Lc) at 10 μ M for 16 hours. The lysate was subjected to electrophoresis on a 4-20% SDS-PAGE gel, then electrophoretically transferred to PVDF membranes. The smear detected above the 200kDa standard represents accumulations of ubiquitinated proteins in the Lc treated cells. The prominent band at ~8kDa corresponds to monoubiquitin.



Immunofluorescent analysis of HeLa cells stained with rabbit pAb to ubiquitin, RPCA-Ubi, dilution 1:1,000 in red, and costained with chicken pAb to vimentin, CPCA-Vim, dilution 1:10,000, in green. The blue is DAPI staining of nuclear DNA. [A] Control HeLa cells maintained in normal medium, [B] HeLa cells treated with 10 μ M of the proteasome inhibitor lactacystin (Lc) for 24 hours. Proteasomal inhibition leads to formation of strongly ubiquitin positive cytoplasmic inclusions. Note the diffuse cytoplasmic ubiquitin staining in control cells and well defined ubiquitin positive inclusions in the Lc treated cells.

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, RPCA-Ubi, was made against purified bovine blood derived ubiquitin coupled to keyhole limpet hemocyanin with glutaraldehyde (6). The antibody 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. We also supply a widely used mouse monoclonal antibody to ubiquitin, MCA-Ubi-1. This antibody binds a peptide which 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.

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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.