Polyubiquitin (K63-linkage-specific) monoclonal antibody (HWA4C4)

Key antibody for the detection of K^{63}-linked polyubiquitinylated proteins.

Modification of proteins by addition of K^{63}-linked polyubiquitin chains is implicated in a variety of cellular events, including DNA repair, signal transduction and receptor endocytosis.

A monoclonal antibody (mAb) has been generated that specifically recognizes K63-linked polyubiquitin chains, but not any other isopeptide-linked (K^6, K^{11}, K^{27}, K^{29}, K^{33}, or K^{48}) polyubiquitin chain or monoubiquitin. The specificity of this K^{63}-linked ubiquitinspecific monoclonal antibody was demonstrated using synthetic ubiquitin chains, single isopeptide-linked polyubiquitin chains, and with endogenously ubiquitinylated species in both cell lysates and intact cells.

The hybridoma secreting the antibody [clone HWA4C4] was generated by fusion of splenocytes from Balb/c mice which had received repeated immunisation with a synthetic peptide encompassing the ubiquitin K^{63}-isopeptide motif. Immunoglobulin was purified from tissue culture supernatant by ion exchange chromatography.

This antibody is covered by our Worry-Free Guarantee.

Citations: 44 View Online »

Ordering Information

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BML-PW0600-0025	25µl
BML-PW0600-0100	100µl

Manuals, SDS & CofA

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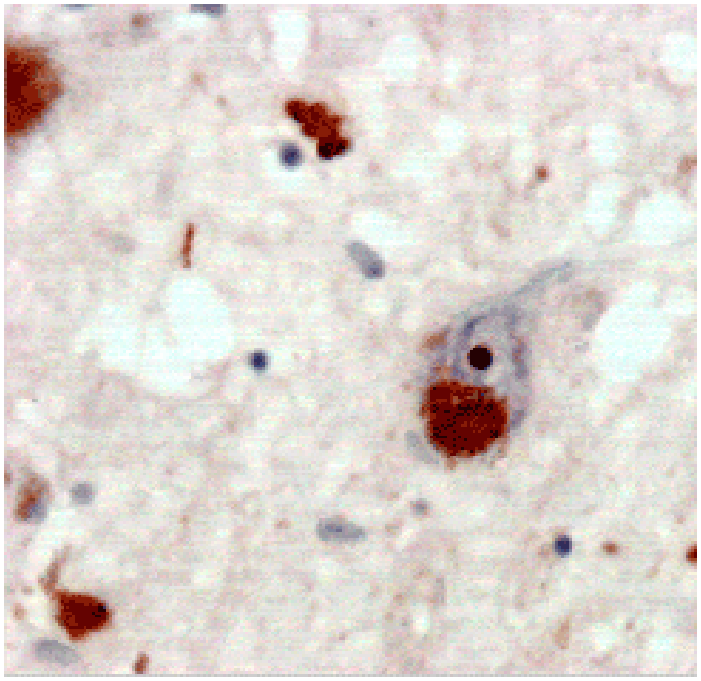


Figure 3: Formalin-fixed paraffin-embedded sections of hippocampus from human Alzheimer's disease brain stained with MAb to Polyubiquitin (K^{63}-linkage-specific) (HWA4C4) at 1:100 dilution with biotinylated secondary antibody and DAB detection.

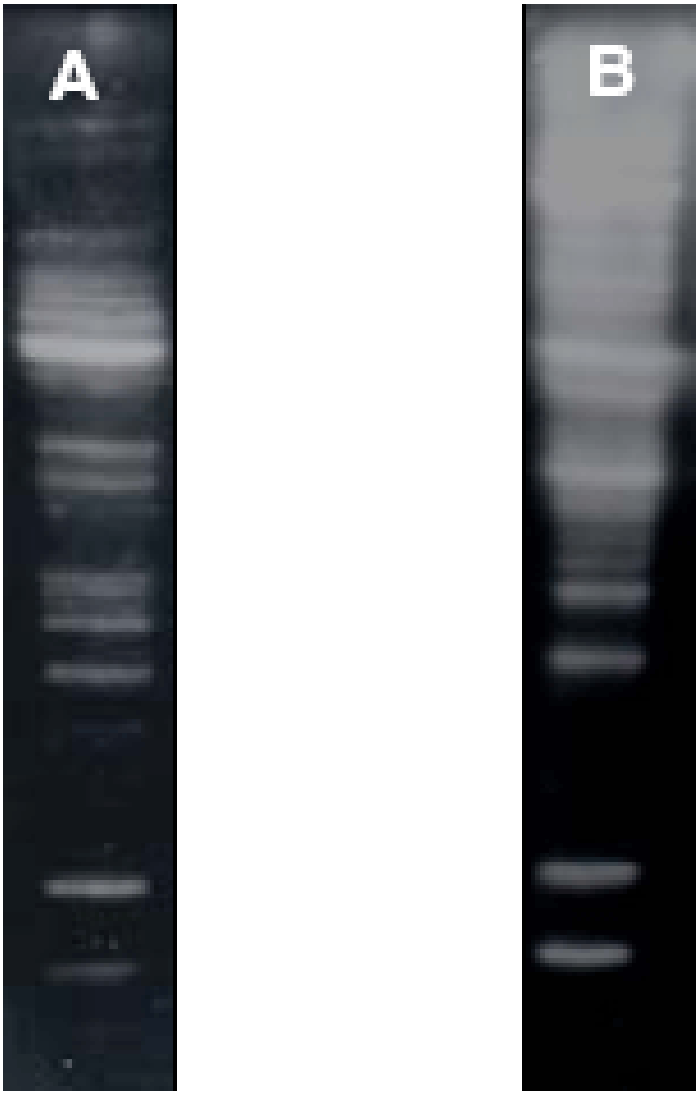


Figure 2: Western blot of HeLa S100 Fraction (BML-SW8750, 5µg) probed with MAb to Polyubiquitin (K^{63}-linkage-specific) (HWA4C4) (BML-PW0600) (Lane A) and MAb to Polyubiquitinylated Conjugates (FK1) (BML-PW8805) (Lane B) at 1:1'000 dilution.

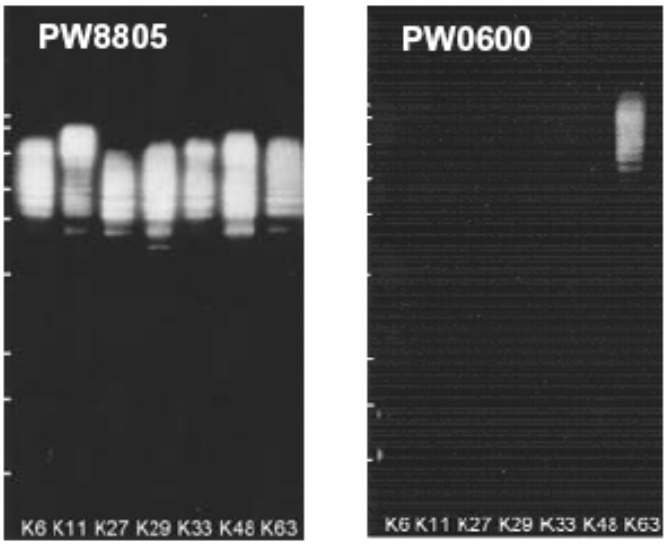


Figure 1: Western blot of single isopeptide-linkage polyubiquitinylated conjugates K^{6} (BML-UW0615), K^{11} (BML-UW0620), K^{27} (BML-UW0625), K^{29} (BML-UW0630), K^{33} (BML-UW0635), K^{48} (BML-UW0640), K^{63} (BML-UW0645),1µg each, using MAb to Polyubiquitinylated Conjugates (FK1) (BML-PW8805) and MAb to Polyubiquitin (K^{63}-linkage-specific) (HWA4C4) (BML-PW0600) at 1:1'000 dilution.

Handling & Storage

Handling	Avoid freeze/thaw cycles. After opening, prepare aliquots and store at -20°C.
Long Term Storage	-20°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application	ELISA, ICC, IHC, WB
Clone	HWA4C4
Crossreactivity	Does not cross-react with any other isopeptide-linked (K6, K11, K27, K29, K33, or K48) polyubiquitinylated species.
Formulation	Liquid. In PBS containing 0.09% sodium azide.
Host	Mouse
Immunogen	Synthetic peptide containing the ubiquitin K63-isopeptide motif.
Isotype	IgG2a
Recommendation Dilutions/Conditions	Immunocytochemistry (1:100)Immunohistochemistry (1:100)Western Blot (1:500 – 1:1,000)Specificity for K63 of mAb to Polyubiquitin (K63-linkage-specific) (HWA4C4) is temperature sensitive. Western blot membranes must not be autoclaved. Excessive heat should be avoided in all applications. K63-linked polyubiquitin modified proteins comprise only a proportion of total cellular polyubiquitinylated species. Increased sample loading may therefore be required for analysis by Western blot. Do not use milk as blocking solution. Use 1% BSA in an appropriate buffer instead.Suggested dilutions/conditions may not be available for all applications.Optimal conditions must be determined individually for each application.
Source	Purified from hybridoma tissue culture supernatant.
Species Reactivity	Species independent

Specificity	Recognizes K^{63}-linked polyubiquitin in wide range of species.
UniProt ID	P0CG47 (UBB), P0CG48 (UBC)
Worry-free Guarantee	**This antibody is covered by our Worry-Free Guarantee .**

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