Proteasome 20S α1, 2, 3, 5, 6 & 7 subunits monoclonal antibody (MCP231)

The proteasome is widely recognised as the central enzyme of non-lysosomal protein degradation. It is responsible for intracellular protein turnover and it is also critically involved in many regulatory processes and, in higher eukaryotes, in antigen processing. The 26S proteasome is the key enzyme of the ubiquitin/ATP-dependent pathway of protein degradation. The catalytic core of this unusually large (2000kDa, 450Å in length) complex is formed by the 20S proteasome, a barrel shaped structure shown by electron microscopy to comprise of four rings each containing seven subunits. Based on sequence similarity, all fourteen 20S proteasomal subunit sequences may be classified into two groups, α and β, each group having distinct structural and functional roles. The α-subunits comprise the outer rings and the β-subunits the inner rings of the 20S proteasome. Observations of the eukaryotic proteasome and analysis of subunit sequences indicate that each ring contains seven different subunits (α7β7β7α7) with a member of each sub-family represented in each particle. Each subunit is located in a unique position within the α- or β-rings. 120S Proteasomes degrade only unfolded proteins in an energy-independent manner, whereas 26S proteasomes degrade native and ubiquitinylated proteins in an ATP-dependent manner. The native protein substrates are recognised by subunits, some with ATP binding sites, of the outer 19S caps of the 26S proteasome. The hybridoma secreting the antibody to subunits HC2, HC3, HC8, HC9, Iota and Zeta was generated by fusion of spenocytes from Balb/c mice which had recieved repeated immunisation with dinitrophenylated proteasomes.

This antibody is covered by our Worry-Free Guarantee.

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Ordering Information

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BML-PW8195-0025	25µl
BML-PW8195-0100	100µl

Manuals, SDS & CofA

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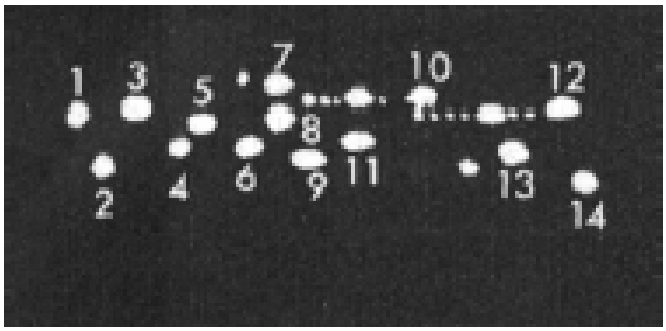


Figure: Ponceau S staining of human placental proteasome preparation after 2D PAGE followed by blotting onto nitrocellulose.

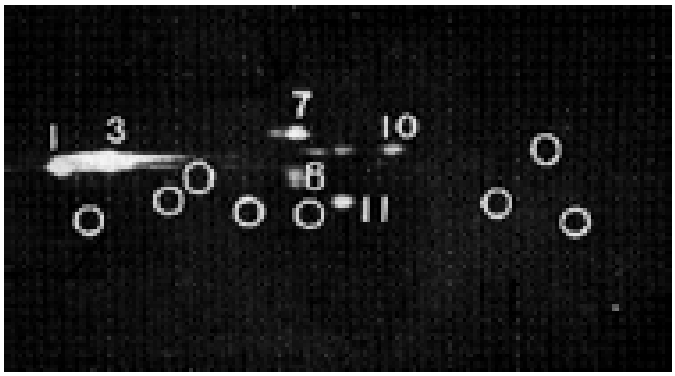
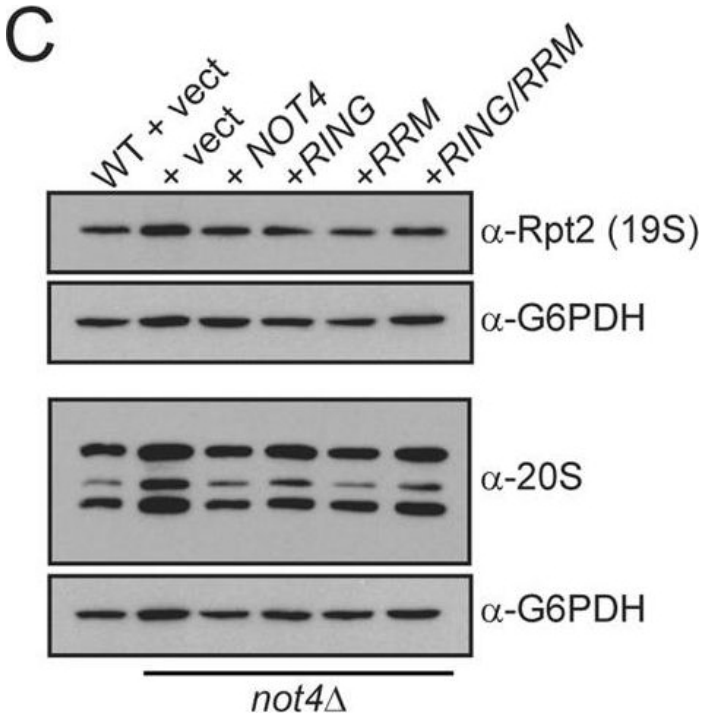


Figure: Luminograph of human placental proteasome preparation after 2D PAGE followed by blotting onto nitrocellulose and probing with antibody Prod. No. BML-PW8195 (MCP231). Antibody dilution 1:1000 using ECL procedure (1 min exposure).



Not4 proteostasis regulation requires the RRM-C domain. (A) Log phase extracts were prepared from the indicated strains under denaturing conditions, extracts were resolved by 7.5% SDS-PAGE, and then immunoblotted with α-ubiquitin. Blots were stripped and re-probed with α-FLAG and α-G6PDH to control for loading, and the total ubiquitin signal was quantified and normalized to G6PDH levels. Data are representative of four independent experiments. Note that because not4Δ control vector cells consistently express higher amounts of G6PDH, the quantified results underestimate the true increase in global polyubiquitylation in this sample. (B) Cell extracts from the indicated strains were prepared, incubated with the LLVY-AMC fluorescent substrate, and fluorescence quantified using a fluorescent plate reader. Triplicate independent extracts were analyzed per sample and the average and SD are plotted. Statistical significance was determined by pairwise (relative to Not4WT) two-sided Student's t-test. **p < 0.01; ***p < 0.005; ****p < 0.001. (C) Immunoblot of extracts from (B) using α-Rpt2 (a 19S proteasome subunit) or α-20S. Note that the α-20S antibody recognizes multiple 20S subunits that have nearly identical mass so their signals overlap. (D) Immunoblot analysis of H3K4me3 and total H3 from the indicated strains. The H3K4me3 and H3 signals were quantified and expressed as a ratio. Data are representative of a minimum of four independent experiments. (E) Equal numbers of cells were 5-fold serially diluted and spotted to control media or media containing 0.5 mg/mL azetidine-2-carboxylic acid (AZC) and incubated at 30 °C for six days. (F) As in (E), except cells were incubated on control media or media containing 0.05 μg/mL cycloheximide for two days.

Handling & Storage

Use/Stability	Dilute in PBS pH 7.2-7.4 and 1% normal goat serum (if a goat anti-mouse IgG linker antibody is to be used)
Handling	Store unopened vial at -20°C until required for use. Aliquot after opening and store at 2-4°C. The use of high quaity \'antiserum-grade\' plastic or glass vials is recommended. Use within 1 month. Avoid freeze/thaw cycles.
Long Term Storage	-20°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Proteasome subunit α type-1, -2, -3, -4, -5 & -6, Macropain subunits C2, C3, C8, C9, ι & ζ
Application	IHC, WB
Clone	MCP231
Formulation	Liquid. In PBS containing 0.01% sodium azide.
Host	Mouse
Immunogen	Dinitrophenylated proteasomes.
Isotype	IgG1κ
Purity Detail	Protein G affinity purified.
Source	Purified from ascites.
Species Reactivity	Human, Mouse, Potato, Rabbit, Rat, Yeast
Specificity	Recognizes the α1, 2, 3, 5, 6 & 7 subunits of the 20S proteasome.
Technical Info / Product Notes	Various systems for the nomenclature of the proteasome subunits have been established. This may be a source of confusion as the system on UniProt differs from “standard” nomenclature as described in the literature. The UniProt ID and Gene Name will help to clearly identify the proteins.

UniProt ID	P25786 (human PSMA1), P25787 (human PSMA2), P25788 (human PSMA3), P25789 (human PSMA4), P28066 (human PSMA5), P60900 (human PSMA6)
Worry-free Guarantee	**This antibody is covered by our Worry-Free Guarantee.**

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