

# Proteasome 19S Rpt5/S6a subunit monoclonal antibody (TBP1-19)

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,  $\alpha$  and  $\beta$ , each group having distinct structural and functional roles. The  $\alpha$ -subunits comprise the outer rings and the  $\beta$ -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 ( $\alpha_7\beta_7\beta_7\alpha_7$ ) with a member of each sub-family represented in each particle. Each subunit is located in a unique position within the  $\alpha$ - or  $\beta$ -rings. In addition to the 20S particle, the 26S complex contains over twenty additional proteins, ranging in molecular weight from 25 to 10kDa, located in a distinct complex called the 'PA700 proteasome activator' or the '19S complex', and which determines substrate specificity and provides the multiple enzymatic functions necessary for proteolysis and viability. Systematic analysis of the sub-unit components have revealed at least six members to be ATPases belonging to a new family of ATP-binding proteins, together with a further fifteen subunits that lack the capacity to bind ATP, isopeptidases and several other proteins thought to be responsible for the unfolding of a protein substrate prior to insertion into the proteolytic core of the 20S proteasome.

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Citations: 29

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## Ordering Information

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ML-PW8770-0025

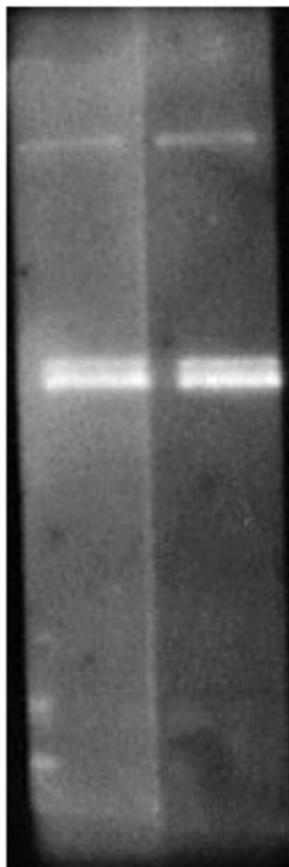
25 $\mu$ l

BM<sup>L</sup>-PW8770-0100

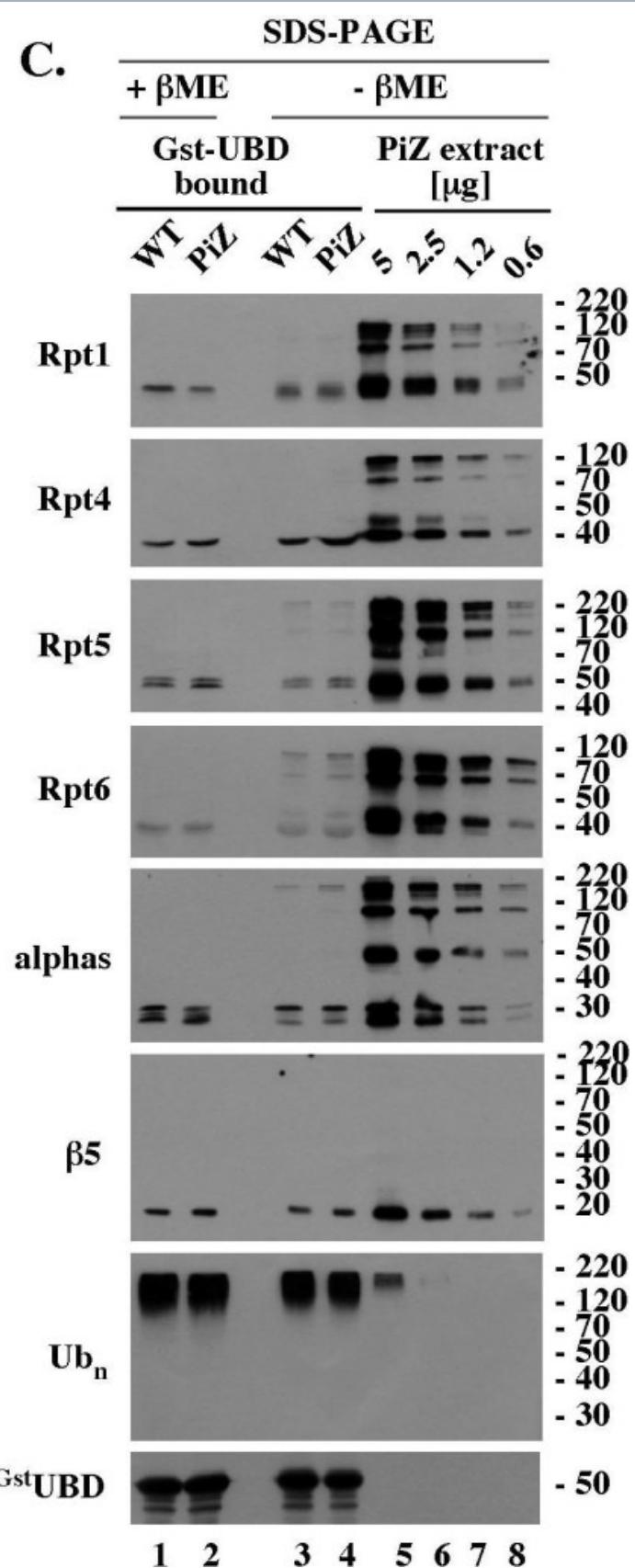
100 $\mu$ l

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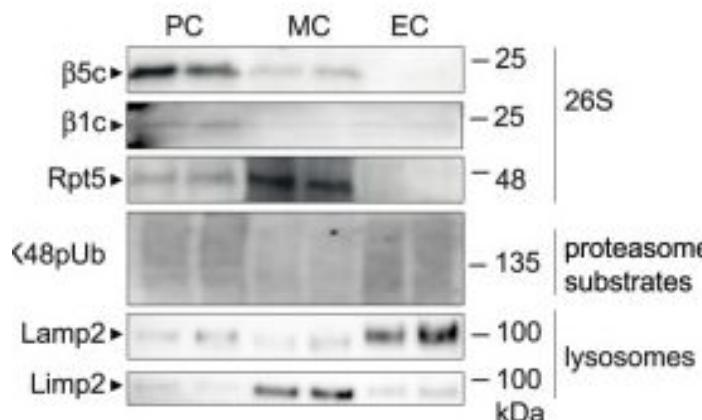


Western blot analysis: Luminograph of rabbit 26S proteasome preparation after SDS PAGE followed by blotting onto PVDF membrane and probing with antibody BML-PW8770. Antibody dilution 1:1000 and 1:2500 using ECL procedure (1 min exposure).



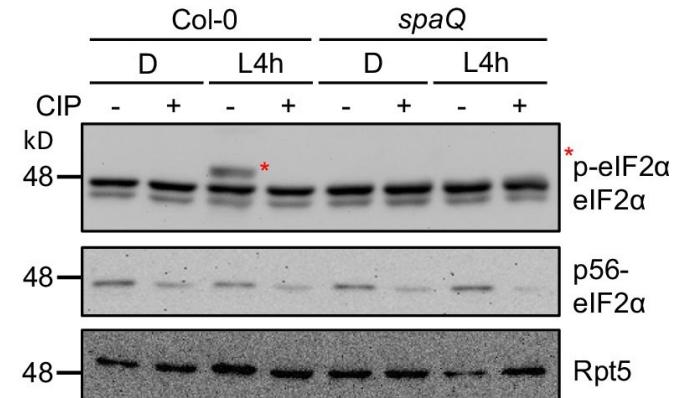
Reduction-sensitive modifications typical of aging WT mice accumulate prematurely on selected proteasomal subunits in the livers of PiZ mice. (A). Rpt4 Western blot analysis of unreduced, un-boiled liver extracts. 5  $\mu$ g of the indicated extracts were mixed with Laemmli buffer without (unreduced samples) or with (reduced samples)  $\beta$ ME (-/+  $\beta$ ME), separated by SDS-PAGE without prior boiling, and analyzed by Western blot with antibodies specific to Rpt4. (B). Rpt4 Western blot analysis of unreduced, but boiled, samples. Experiment like in A, lanes 1–9, except that extracts were mixed with Laemmli buffer without  $\beta$ ME (-  $\beta$ ME). Unreduced (lanes 1–4) and reduced (lanes 5–8) samples were boiled for 5 min before SDS-PAGE analysis.

F



Glomerular cell-specific distribution of proteasomal and lysosomal proteins in human and murine glomeruli. A Schematic depiction of the ubiquitin proteasome system (UPS) and autophagosome-lysosome pathway (ALP) with individual marker proteins used for the expression analyses within glomerular cell types. Ubiquitin = polypeptide involved in dynamic regulation of protein function, localization, and stability; Rpt5 = proteasome regulatory subunit 6A (Psmc3) of the proteasome 19S regulatory particle;  $\beta 5c$  (Psmb5) = main proteolytic subunit of the constitutive 20S core particle; LC3 = microtubule-associated protein 1A/1B-light chain 3; Lamp1 and Lamp2 = lysosomal-associated membrane proteins 1 or 2; Limp2 (Scarb2) = lysosomal integral membrane protein 2. Distribution of marker proteins (green) by high-resolution confocal images in a healthy human (B) and murine (C) glomerulus in relation to the slit diaphragm protein nephrin (red) and DNA (blue); pc podocyte, mc mesangial cell, ec glomerular endothelial cell, pec parietal epithelial cell, white arrows point toward endothelial lining filled with Lamp2-positive lysosomes,  $n = 3$  individuals. D–F Podocytes (PC), mesangial cells (MC) and glomerular endothelial cells (EC) were bulk-isolated from glomeruli. D Proteomic analyses depict molecular properties of glomerular cell types as shown by the radar plot, whereby two-fold changes of distinct uniprot key words are plotted. Protein values were obtained by label-free quantification results using the MaxQuantLFQ algorithm<sup>72</sup>. E Relative transcript levels quantified via qRT-PCR of Psmb5 (encoding for  $\beta 5c$ ) and Scarb2 (encoding for Limp2) normalized to 18S as home keeper in relation to total glomerular transcript levels (dashed line), mean  $\pm$  SEM,  $*p = 0.0292$  (PC Psmb5),  $*p = 0.048$  (MC Scarb2), one-way ANOVA with Bonferroni post-test for multiple comparisons,  $n = 12$  of 2 pooled independent experiments. F Protein abundance from isolated glomerular cell types determined by immunoblot, equal loading was ensured by loading equal numbers of EACG control (PC), MC, and EC,  $n = 3$  independent experiments.

d



SPA1 phosphorylates C-terminus of eIF2 $\alpha$ .1 and eIF2 $\alpha$ .2 in a light-dependent manner. a Predicted phosphorylation sites located at C-terminal eIF2 $\alpha$  are indicated in red. Tyr, Ser or Thr were then replaced with alanine (A) and aspartic acid (D) to generate a phospho-null form and a phospho-mimicking form of eIF2 $\alpha$ , respectively. b A semi-in vivo kinase assay showed that SPA1 protein extracted from TAP-SPA1 overexpression line phosphorylates wild-type (WT) eIF2 $\alpha$  but not the phospho-null form (6A/8A) of eIF2 $\alpha$  (autoradiogram in upper panel). The plant extracts were derived from 4-day-old TAP-SPA1 seedlings incubated either under dark (D) or illuminated under white light for 4 h (L4h) before sampling. Three biological repeats of data showed the same results. c A semi-in vivo kinase assay showed that WT version of eIF2 $\alpha$  has higher phosphorylation intensity in TAP-SPA1 than in spaQ under light condition (autoradiogram in upper panel). The plant extracts were derived from 4-day-old TAP-SPA1 and spaQ incubated either under dark (D) or illuminated with 4 h white light (L4h) before sampling. The GST only was used as negative control. The lower panel shows the protein levels in a Coomassie blue staining gel. Three biological repeats of data showed the same results. d Immunoblots showed phosphorylation of eIF2 $\alpha$  under both dark and light condition in Col-0 and spaQ. Four-day-old Col-0 and spaQ etiolated seedlings incubated either under dark (D) or illuminated with 4 h white light (L4h) before sampling, and extracted proteins were then separated on 6.5% sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) gels containing 15  $\mu$ M Phos-tag. The slow-migrating band (\*) is the phosphorylated form of eIF2 $\alpha$ . Three biological repeats of data showed the same results. Treatment of calf intestinal alkaline phosphatase (CIP, +) and inactivated boiled CIP (-) were shown. Rpt5 proteins were used as loading control.

Image collected and cropped by CiteAb under a CC-BY license from the following publication: The phosphorylation of carboxyl-terminal eIF2 $\alpha$  by SPA



## Handling & Storage

**Long Term Storage** -20°C

**Shipping** Blue Ice

## Regulatory Status

RUO - Research Use Only

## Product Details

<b>Alternative Name</b>	TBP1, Tat-binding protein 1, 26S protease regulatory subunit 6A, Proteasome 26 S subunit ATPase 3
<b>Application</b>	IHC, WB
<b>Clone</b>	TBP1-19
<b>Formulation</b>	Liquid. Contains 10mM sodium azide.
<b>Host</b>	Mouse
<b>Immunogen</b>	Recombinant full-length human Rpt5.
<b>Isotype</b>	IgG2b
<b>Purity Detail</b>	Partially purified ascites.
<b>Source</b>	Purified from hybridoma tissue culture supernatant.
<b>Species Reactivity</b>	Human, Mouse, Rabbit, Rat
<b>Specificity</b>	Recognizes the Rpt5/S6a subunit of the 19S regulatory subunit

The hybridoma secreting the antibody to subunit Rpt5/S6a was generated by fusion of splenocytes from Balb/c mice that had received repeated immunisation with a full length human recombinant protein with Sp2/0-Ag14 myeloma cells. The antibody (clone TBP1-19) has been characterised by one-dimensional Western blotting.

Immunoblotting – Single dimension SDS-PAGE of a purified rabbit 26S proteasome preparation followed by Western blotting at optimal dilution (1:2500) gives a major band with a relative molecular weight of approximately 50kDa. There is a minor band observed running slightly slower that may be due to modification.

Immunoprecipitation – This antibody has not been characterised for this purpose.

Immunohistochemistry – This antibody has been shown to be suitable for this purpose in the subcellular localisation of proteasomes.

**UniProt ID**

P17980

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