

Product name(s):	Anti-19S Regulator Non-ATPase Subunit Rpn12 (S14), Monoclonal (clone p31-27)
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Catalogue number:	PW 8835	Batch number:	Z02896	Expiry date:	12 months upon receipt
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Product information:

The hybridoma secreting the antibody to subunit Rpn12 was generated by fusion of splenocytes from Balb/c mice that had received repeated immunisation with recombinant human Rpn12 protein. The antibody (clone p31-27) has been extensively characterised by one- and two-dimensional Western blotting. The immunoglobulin subclass is IgG₁. Vial contains a partially purified immunoglobulin preparation containing 0.01M sodium azide.

Application data:

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 ($\alpha_7\beta_7\beta_7\alpha_7$) with a member of each sub-family represented in each particle. Each subunit is located in a unique position within the α - or β -rings¹.

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 sub-units 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 proteasome².

Data:

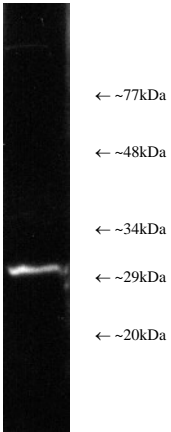
Subunit Rpn12; alternative names: S14, p31 (human); GDB ref: PSMD8; Swiss-Prot accession number: P48556; length: 257 amino acids; molecular weight: 30004.; theoretical pI 6.85.

Immunoblotting - Single dimension SDS-PAGE of recombinant S14 protein and HeLa cell lysates shows a single band with a relative molecular weight of approximately 30kDa. There is no cross-reactivity with any of the other 26S proteasome 'lid' subunits. An initial titre of 1:1000 is recommended.

Immunoprecipitation - This antibody has not been characterised for use in immunoprecipitation.

Immunohistochemistry - This antibody has not been characterised for use in immunohistochemistry.

Species reactivity:- This antibody has been shown to react with human protein. No data is currently available for other species.



← ~77kDa
← ~48kDa
← ~34kDa
← ~29kDa
← ~20kDa

Luminograph of HeLa cell cytosolic preparation after SDS PAGE followed by blotting onto PVDF membrane and probing with antibody PW 8835. Antibody dilution 1:5000 using ECL procedure (1 min exposure).

Storage and use:

Store unopened vial at -20°C until required for use. AVOID REPEATED FREEZE-THAW CYCLES. Aliquot undiluted antibody into smaller volumes (not less than 10µL) prior to freezing if appropriate. The use of high quality 'antiserum-grade' plastic or glass vials is recommended. Store diluted antibody at 2-4°C (do not freeze) and use within 1 month. Dilute to working strength with phosphate buffered saline pH 7.2-7.4.

Background references:

- Kopp, F., Hendil, K.B., Dahmann, B., Kristensen, P., Sobek, A. and Uerkvitz, W. Subunit arrangement in the human 20S proteasome. *Proc. Natl. Acad. Sci. USA*, **94**, 2939-2944 (1997).
- Tanaka, K. and Tsurumi, C. The 26S proteasome: subunits and functions. *Molecular Biology Reports*, **24**, 3-11 (1997).