PRODUCT DATA SHEET

Product name(s):	Anti-19S Regulator Non-ATPase Subunit Rpn8 (S12), Polyclonal

Catalogue number:	PW 8180	Batch number:	Z02616	Expiry date:	10/2004

Product information:

The polyclonal antibody to Rpn8 was generated by immunisation of sheep with a recombinant protein corresponding to residues 1-205 of the human Rpn8. The antibody has been characterised by one-dimensional SDS PAGE/Western blotting. Vial contains an immunoglobulin preparation suspended in phosphate-buffered saline 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-rings[1].

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 proteasome[2, 3].

Data:

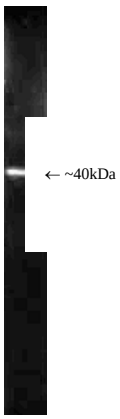
Subunit Rpn8; alternative names: 26S proteasome non-ATPase subunit 7, S12 (human), Mov34 (mouse); GDB ref: PSMD7; chromosomal locus: 16q22.2; Swiss-Prot accession number: P51665; length: 324 amino acids; molecular weight: 37060.; theoretical pI 6.11.

Immunoblotting - Single dimension SDS-PAGE of recombinant S12 fragments (residues 1-95 and 1-205) as well as the full length protein gives single bands with a relative molecular weight of approximately 40kDa. Similarly, a human placental proteasome preparation gives a band at a relative molecular weight of ~40kDa.

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

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

Species reactivity:- This antibody has been shown to react with human proteins.



Luminograph of human placental proteasome preparation after SDS PAGE followed by blotting onto PVDF membrane and probing with antibody PW 8180. Antibody dilution 1:1000 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 and 1% normal rabbit serum (if a rabbit anti-sheep IgG linker antibody is to be used).

References:

1. Kopp, F., Hendil, K.B., Dahlmann, B., Kristensen, P., Sobek, A. and Uerkvitz, W. Subunit arrangement in the human 20S proteasome. *Proc. Natl. Acad. Sci. USA*, **94**, 2939-2944 (1997).
2. Tanaka, K. and Tsurumi, C. The 26S proteasome: subunits and functions. *Molecular Biology Reports*, 24, 3-11 (1997).
3. Dubiel, W., Ferrell, K. and Rechsteiner, M. Subunits of the regulatory complex of the 26S protease. *Molecular Biology Reports*, 21, 27-34 (1995).