

PRODUCT DATA SHEET



Revised: 06 October 2004
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Product name(s):	PA28 Activator [11S Activator] (human)				
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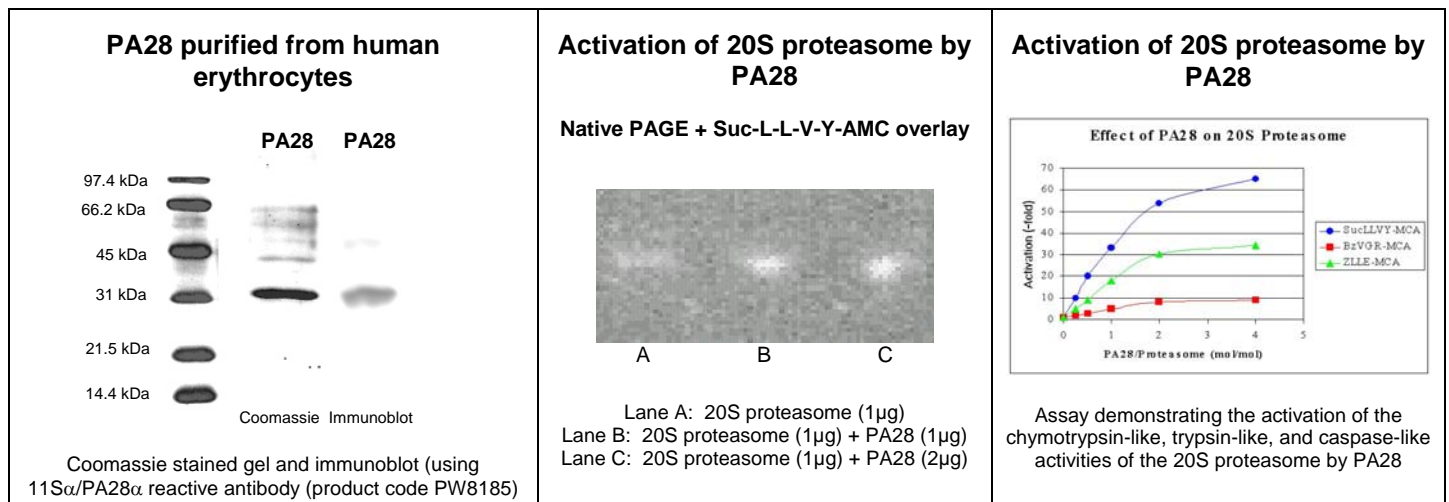
Catalogue number:	PW 9420	Batch number:	Z0xxxx	Expiry date:	3 months from receipt
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Product information:

The proteasome activator PA28, or 11S regulator, is a protein complex composed of two different but homologous polypeptides, termed PA28 α and PA28 β . The purified activator protein (with a native molecular weight of approximately 200,000) is a ring-shaped containing the two polypeptides, with a stoichiometry of $\alpha_3\beta_3$ or $\alpha_4\beta_3$, which are about 50% identical in primary structure, and which have been shown to be part of the same protein complex¹. Whilst the hexameric structure appears favoured, there remains debate as to the exact stoichiometry with some crystal data and equilibrium sedimentation experiments indicating that PA28 forms a heptameric ring².

PA28 activates hydrolysis of small non-ubiquitinated peptides by the 20S proteasome; however, alone it shows no hydrolytic activity eliciting activation of the proteasome's multiple peptidase activities by binding to the terminal α -rings of the 20S core particle. PA28 is inactivated by treatment with carboxypeptidase Y, which cleaves Tyr and Ile residues from the carboxyl terminus of the α -subunit but which has very little effect on the β -subunit. This indicates that a short carboxyl-terminal sequence of the α -subunit is critical for binding of native PA28 to the proteasome¹ whilst the β -subunit modulates PA28 activity, perhaps by influencing the affinity of PA28 for the proteasome³. PA28 modulates the proteasome-catalysed production of antigenic peptides presented to the immune system on MHC class I molecules indicating a cellular function of the activator in antigen processing⁴.

Due to the intrinsic instability of the PA28 complex, it is extremely difficult to obtain naturally sourced human PA28 in a state of high purity whilst retaining its ability to efficiently activate latent 20S proteasome. This new product has been prepared in such a way that ensures that both criteria are met from a practical point of view. The absolute protein purity is estimated to be of the order of 70% whilst a typical PA28 concentration to activate latent 20S proteasome is 1 to 4 times that of the molar concentration of 20S proteasome. For example, if using 0.7pmol (0.5 μ g) 20S proteasome for assay, add 1.5 – 3.0pmol (0.4–0.8 μ g) PA28 for activation. At this ratio one might expect to see an increase of activation of the 20S proteasome of the order of 5-20 fold depending on the extent of latency of the enzyme preparation and the substrate used. The peptidic activities of the 20S proteasome may be demonstrated using peptide substrates such as Suc-LLVY-AMC (product code P-802) as shown below.



Stability, storage and specific hazard data:

The complex is supplied in buffer comprising 50%(v/v) glycerol/0.5mM EDTA/0.5mM NaN₃/0.5mM DTT/10mM Tris/HCl, pH7.5 at a concentration of 50 μ g/mL. The material should be stored at -80°C and once thawed should not be refrozen as the activity will be lost if subject to multiple freeze/thaw cycles. Activity will be retained for several days if the material is stored on ice.

References:

1. Song, X. L., Mott, J. D., Vonkampen, J., Pramanik, B., Tanaka, K., Slaughter, C. A., and De Martino, G. N. A model for the quaternary structure of the proteasome activator PA28. *Journal of Biological Chemistry*, **271**, 26410-26417 (1996).
2. Johnston, S. C., Whitby, F. G., Realini, C., Rechsteiner, M., and Hill, C. P. The proteasome 11s regulator subunit REG-alpha (PA28-alpha) is a heptamer. *Protein Science*, **6**, 2469-2473 (1997).
3. Song, X. L., Vonkampen, J., Slaughter, C. A., and Demartino, G. N. Relative functions of the alpha and beta subunits of the proteasome activator, PA28. *Journal of Biological Chemistry*, **272**,

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4. 27994-28000 (1997).
Kuehn, L. and Dahlmann, B. Structural and functional properties of proteasome activator PA28. *Molecular Biology Reports*, 24(1-2), 89-93, (1997).