S5a [untagged]
Substrate

Alternate Names: PSMD4, RPN10, 26S proteasome non-ATPase regulatory subunit 4, MCB1

Cat. No. **66-2002-050**
Lot. No. **30177**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

The ubiquitin–proteasome system (UPS) targets selected proteins for degradation by the 26S proteasome. The initial steps in this pathway generate proteins that are covalently tagged with a polyubiquitin chain that is then recognized by ubiquitin receptors of the 26S proteasome. This is a large complex composed of a 20S catalytic core particle and two 19S regulatory particles (Kok *et al.,* 1993) that catalyse the final step in the pathway. While the 20S particle comprises a catalytic chamber for protein degradation, collectively the proteins that compose the 19S particle perform several functions that include recognition of ubiquitylated substrates, cleavage of the polyubiquitin chain for ubiquitin recycling, control of access to the 20S proteolytic chamber, and substrate unfolding and subsequent translocation into the 20S core particle for degradation (Boehringer *et al.,* 2012). S5a (Rpn10) is a major ubiquitin binding protein that binds preferentially to polyubiquitin chains. It is found as a subunit of the 26S proteasome, but unlike other proteasome subunits, S5a also exists predominantly as a free protein in the cytosol. S5a contains two stretches of approximately 15 amino acids called the ubiquitin interacting motifs (UIMs), which are responsible for its high affinity for ubiquitin chains (Kim and Goldberg, 2012). One essential feature of the UPS is that the proteasome must have the ability to capture substrates by recognizing their covalently linked polyubiquitin chains. S5a and Rpn13 are two major such subunits of the 19S regulatory particle (RP). The polyubiquitin on a substrate can bind with S5a through its C-terminal UIMs, or with Rpn13 through its N-terminal pleckstrin-like receptor for Ub (Pru) domain. Recently it was proposed that polyubiquitin

Continued on page 2

Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5,
150 mM sodium chloride,
2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~41 kDa

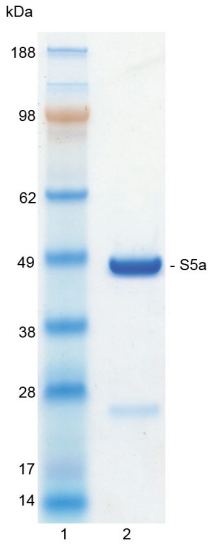
Purity: >85% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

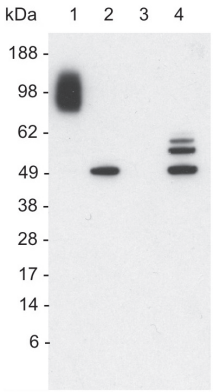
Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg S5a



Protein Identification:
Confirmed by mass spectrometry.

Substrate Ubiquitylation Assay:
Incubation of S5a for 1 hour at 37˚C in the presence of His-UBE1, His-UBE2W, His-UBE2N and His-UBE2V1, CHIP, ubiquitin and ATP (Lane 1) was compared alongside three control reactions with either ATP (Lane 2), S5a (Lane 3) or CHIP (Lane 4) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an anti-S5a antibody and these were observed only in the presence of both ATP and S5a. A degree of E3 (CHIP)-independent ubiquitylation of S5a is also apparent (compare Lane 4 with Lanes 1 and 2).



UBIQUIGENT™
www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

S5a [untagged]
Substrate

Alternate Names: PSMD4, RPN10, 26S proteasome non-ATPase regulatory subunit 4, MCB1

Cat. No.	**66-2002-050**	Quantity:	50 µg
Lot. No.	**30177**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

Background

Continued from page 1

can bind with S5a and Rpn13 simultaneously (Elangovan *et al.,* 2010). S5a is also itself regulated by ubiquitylation. Monoubiquitylation of S5a has been shown to strongly inhibit the capacity of S5a to interact with substrates, thus decreasing proteasome activity (Isasa *et al.,* 2010). S5a is commonly used as a control substrate because unlike typical substrates of E3 ligases, S5a can be ubiquitylated by all types of E3s tested including the multimeric and monomeric Ring finger E3s (MuRF1, Siah2, Parkin, APC, and SCFTRCP1), the U-box E3, CHIP, and HECT domain E3s (E6AP and Nedd4) when assayed with the Ube2D family of E2 conjugating enzymes (Kim *et al.,* 2009; Uchiki *et al.,* 2009).

References:

Boehringer J, Riedinger C, Paraskevopoulos K, Johnson EO, Lowe ED, Khoudian C, *et al.* (2012) Structural and functional characterization of Rpn12 identifies residues required for Rpn10 proteasome incorporation. *Biochem J* **448**, 55-65.

Elangovan M, Oh C, Sukumaran L, Wojcik C and Yoo YJ (2010) Functional differences between two major ubiquitin receptors in the proteasome; S5a and hRpn13. *Biochem Biophys Res Commun* **396**, 425-428.

Isasa M, Katz EJ, Kim W, Yugo V, Gonzalez S, Kirkpatrick DS, *et al.* (2010) Monoubiquitination of RPN10 regulates substrate recruitment to the proteasome. *Mol Cell* **38**, 733-745.

Kim HT and Goldberg AL (2012) S5a/Rpn10, a UIM-protein, as a universal substrate for ubiquitination. *Meth Mol Biol* **832**, 653-660.

Kim HT, Kim KP, Uchiki T, Gygi SP and Goldberg AL (2009) S5a promotes protein degradation by blocking synthesis of nondegradable forked ubiquitin chains. *EMBO J* **28**, 1867-1877.

Kok K, Hofstra R, Pilz A, van den Berg A, Terpstra P, Buys CH, *et al.* (1993) A gene in the chromosomal region 3p21 with greatly reduced expression in lung cancer is similar to the gene for ubiquitin-activating enzyme. *Proc Natl Acad Sci USA* **90**, 6071-6075.

Uchiki T, Kim HT, Zhai B, Gygi SP, Johnston JA, O'Bryan JP, *et al.* (2009) The ubiquitin-interacting motif protein, S5a, is ubiquitinated by all types of ubiquitin ligases by a mechanism different from typical substrate recognition. *J Biol Chem* **284**, 12622-12632.

Physical Characteristics

Continued from page 1

Protein Sequence:

GPLGS***M***VLESTMVCVDNSEYMRNGDFLP
TRLQAQQDAVNIVCHSKTRSNPENNVGLIT
LANDCEVLTTLTPDTGRILSKLHTVQPK
GKITFCTGIRVAHLALKHRQGKNHKMRI
IAFVGSPVEDNEKDLVKLAKRLKKEKVNV
DIINFGEEEVNTEKLTAFVNTLNGKDGTG
SHLVTVPPGPSLADALISSPILAGEGGAML
GLGASDFEFGVDPSADPELALALRVSME
EQRQRQEEEARRAAAASAAEAGIATTGTEDS
DDALLKMTISQQEFGRTGLPDLSSMTEEEQ
IAYAMQMSLQGAEFGQAESADIDASSAM
DTSEPAKEEDDYDVMQDPEFLQSVLENLPGVD
PNNEAIRNAMGSLASQATKDGKKDKKEEDKK

The residues underlined remain after cleavage and removal of the purification tag.
S5a (regular text): Start ***bold italics*** (amino acid residues 1-377)
Accession number: NP_002801

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0