RNF11 [6His-tagged]

E3 Ligase

Alternate Names: ring finger protein 11; CGI-123; SID 1669

Cat. No. **63-0023-025**
Lot. No. **30025**

Quantity: 25 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 1

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). Ring Finger Protein 11 (RNF11) is a member of the E3 protein ligase family and cloning of the human gene was first described by Seki *et al.* (1999). The intrinsic E3 ligase activity of RNF11 is conferred through a RING domain at the C-terminus of the protein. RNF11 has been shown to interact with the HECT-type E3 ubiquitin ligases Nedd4, AIP4, SMURF1 and SMURF2, as well as with Cullin1, the core protein of the multi-subunit SCF E3 ubiquitin ligase complex (Santonico, Belleudi *et al.*, 2010; Kitching *et al.*, 2003). RNF11 has been found to mediate the ubiquitylation of AMSH by the E3 ligase SMURF2. It is thought that RNF11 recruits AMSH to SMURF2 for ubiquitylation, leading to its degradation by the 26S proteasome (Li and Seth 2004).

References:

Kitching, R., M. J. Wong, *et al.* (2003) The RING-H2 protein RNF11 is differentially expressed in breast tumours and interacts with HECT-type E3 ligases. *Biochim Biophys Acta* **1639**(2), 104-12.

Li, H. and A. Seth (2004) An RNF11: Smurf2 complex mediates ubiquitination of the AMSH protein. *Oncogene* **23**(10), 1801-8.

Santonico, E., F. Belleudi, et al.(2010) Multiple modification and protein interaction signals drive the Ring finger protein 11 (RNF11) E3 ligase to the endosomal compartment. *Oncogene* **29**(41), 5604-18.

Seki, N., A. Hattori, et al. (1999) Cloning and expression profile of mouse and human genes, Rnf11/RNF11, encoding a novel RING-H2 finger protein. *Biochim Biophys Acta* **1489**(2-3), 421-7.

Physical Characteristics

Species: human

Source: *E. coli* expression

Quantity: 25 µg

Concentration: 0.5 mg/ml

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

Molecular Weight: ~19.9 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

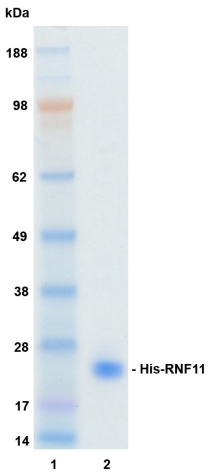
MGSSHHHHHHSSGLEVLFQGPGS***M***GNCLK
SPTSDDISLLHESQSDRASFGEGTEPDQEPP
PPYQEQVPVPVYHPTPSQTRLATQLTEEEQ
IRIAQRIGLIQHLPKGVYDPGRDGSEKKI
RECVICMMDFVYGDPIRFLPCMHIYHLDCID
DWLMRSFTCPSCMEPVDAALLSSYETN

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
RNF11 (regular text): Start ***bold italics*** (amino acid residues 1-154)
Accession number: NP_055187

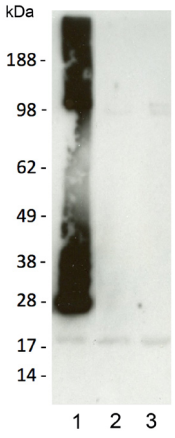
Quality Assurance

Protein Identification:
Confirmed by mass spectrometry.

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-RNF11



E3 ligase assay:
The ubiquitin conjugating activity of His-RNF11 was validated through its ability to catalyse the generation of polyubiquitin chains in the presence of the E1 activating enzyme His-UBE1, the E2 conjugating enzyme His-UBE2D1 (UbcH5a) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of His-RNF11 for 120 minutes at 37˚C in the presence of ubiquitin, His-UBE1, His-UBE2D1 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or His-RNF11 (Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an anti-ubiquitin conjugate antibody and these were observed only in the presence of both ATP and His-RNF11.



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Lot-specific COA version tracker: v1.0.0