

RNF8 [GST-tagged]

E3 Ligase

Alternate Names: C3HC4 type zinc finger protein; KIAA0646; Ring finger protein (C3HC4 type) 8; Ring finger protein 8; UBC13/UEV-interacting ring finger protein

Cat. No. 63-0021-025

Lot. No. 30032

Quantity: 25 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

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 8 (RNF8) is a member of the E3 protein ligase family and cloning of the human gene was first described by Ishikawa *et al.* (1998). RNF8 is required for the ubiquitylation of some nuclear proteins, promoting their subsequent degradation (Kolas *et al.*, 2007). RNF8 has also been shown to interact with the E2 conjugating enzyme Ubc13 (UBE2N) recruiting BRAC1 and 53BP1 to sites of nuclear damage (Kolas *et al.*, 2007; Lok *et al.*, 2011; Santos *et al.*, 2010). RNF8 knockout mice display growth retardation and an increased pre-disposition to cancer (Li *et al.*, 2010).

References:

Ishikawa K, Nagase T, Suyama M, Miyajima N, Tanaka A, Kotani H, Nomura N, Ohara O (1998) Prediction of the coding sequences of unidentified human genes. X. The complete sequences of 100 new cDNA clones from brain which can code for large proteins *in vitro*. *DNA Res* 5, 169-76.

Kolas NK, Chapman JR, et al. (2007) Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 318, 1637-40.

Li L, Halaby MJ, et al. (2010) Rnf8 deficiency impairs class switch recombination, spermatogenesis, and genomic integrity and predisposes for cancer. *J Exp Med* 207, 983-97.

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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: ~83.4 kDa

Purity: >80% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

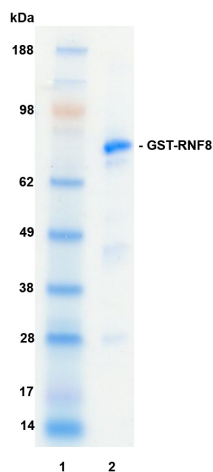
Quality Assurance

Protein Identification:

Confirmed by mass spectrometry.

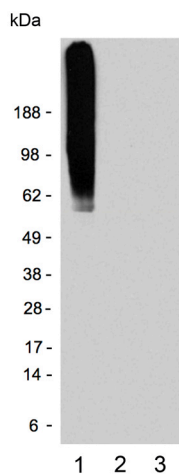
Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-RNF8



E3 ligase assay:

The ubiquitin conjugating activity of GST-RNF8 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-UBE2D4 (UbcH5d) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of GST-RNF8 for 30 minutes at 30°C in the presence of ubiquitin, His-UBE1, His-UBE2D4 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-RNF8 (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 GST-RNF8.



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Lot-specific COA version tracker: v1.0.1

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

Lok GT, Sy SM, Dong SS, Ching YP, Tsao SW, Thomson TM, Huen MS (2011) Differential regulation of RNF8-mediated Lys48- and Lys63-based poly-ubiquitylation. *Nucleic Acids Res.* [Epub ahead of print]

Santos MA, Huen MS, et al. (2010) Class switching and meiotic defects in mice lacking the E3 ubiquitin ligase RNF8. *J Exp Med* 207, 973-81.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTSMAIRYIADKHNLG
GCPKERAEISMLEGAVLDIRYGVSR IAY
SKDFETLKVDFLSKLPEMLKMFEDRLCHK
TYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAI PQIDKYLKSSKY
IAWPLQGWA TFGGGDHPPKSDLEVL FQG
PLGSPEIPGSTRAAAMGEPGFVVTGDR
AGGRSWCLRRVGM SAGWLLLEDGCEVT
VGRGFVVTYQLVSKICPLMISRNHCV
LKQNPEGQWTIMDNKSLNGVWLNRRARLE
PLRVYSIHQGDYIQLGVPLENKENAEY
EYEVTEEDWETIYPCLSPKNDQMI EKN
KELRTRKRKFSLDEL AGPGAEGPSNLK
SKINKVSCESGQPVKSQ GKGEVASTPS
DNLDPKLTALEPSKTTGAPIY PGF
PKVTEVHHEQKASNSSASQ RSLQM
FKVTMSRILRLKIQMQEKHEAVMNVK
KQTQKGN SKKVQMEQELQDLQSQ L
CAEQAAQQARVEQLEKTFQEEEQHLQGLE
IAQGEKDLKQQLAQA LQEHWALMEEEL
NRSKKDFEAI IQAKNKELEQTKEEKEK
MQAQKEEVL SHMNDVLENE LQCIICSEY
FIEAVTLNCAHSFCSYCIN EWMKRKIECPI
CRKDIKSKTYSLVLDN CINKMVNNLSSEVK
ERRIVLIRERKAKRLF

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVL FQ▼GP)
RNF8 (regular text): Start **bold italics** (amino acid residues 1-485)
Accession number: NP_003949



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