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.

Continued on page 2

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

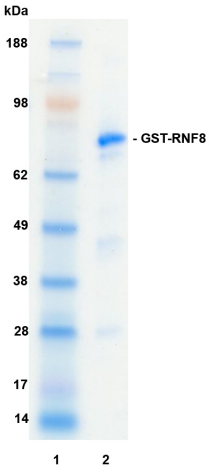
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

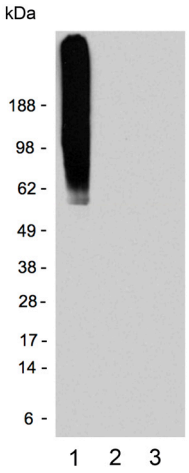
Quality Assurance

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.



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: v1.0.1

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**	Quantity:	25 µg
Lot. No.	**30032**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

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:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPEMLKMFEDRLCHK
TYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAIPQIDKYLKSSKY
IAWPLQGWQATFGGGDHPPKSDLEVLFQG
PLGSPEIPGSTRAAA***M***GEPGFFVTGDR
AGGRSWCLRRVGMSAGWLLLEDGCEVT
VGRGFGVTYQLVSKICPLMISRNHCV
LKQNPEGQWTIMDNKSLNGVWLNRARLE
PLRVYSIHQGDYIQLGVPLENKENAEY
EYEVTEEDWETIYPCLSPKNDQMIEKN
KELRTKRKFSLDELAGPGAEGPSNLK
SKINKVSCESGQPVKSQGKGEVASTPS
DNLDPKLTALEPSKTTGAPIYPGF
PKVTEVHHEQKASNSSASQRSLQM
FKVTMSRILRLKIQMQEKHEAVMNVK
KQTQKGNSKKVVQMEQELQDLQSQL
CAEQAQQQARVEQLEKTFQEEEQHLQGLE
IAQGEKDLKQQLAQALQEHWALMEEL
NRSKKDFEAIIQAKNKELEQTKEEKEK
MQAQKEEVLSHMNDVLENELQCIICSEY
FIEAVTLNCAHSFCSYCINEWMKRKIECPI
CRKDIKSKTYSLVLDNCINKMVNNLSSEVK
ERRIVLIRERKAKRLF

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

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: v1.0.1