ZNRF2 [GST-tagged]

E3 Ligase

Alternate Names: Zinc and ring finger protein 2

Cat. No. **63-0041-025**
Lot. No. **30217**

Quantity: 25 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

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). Zinc and Ring Finger protein 2 (ZNRF2) is a member of the Really Interesting New Gene (RING) E3 protein ligase family and cloning of the human gene was first described by Araki *et al.* (2001). ZNRF2 has been shown to be expressed in both the central and peripheral nervous system of rats during development and in adulthood (Araki *et al.*, 2001). Ube2D2 and Ube2D3 have been identified as supporting E2 conjugating enzymes in the *in vitro* ubiquitylation activity of ZNRF2 and mutation of the Zinc finger Ring finger domain of ZNRF2 showed that it was required for E3 ligase activity of ZNRF2 (Araki and Milbrandt 2003). ZNRF2 also regulates non-canonical ubiquitylation by binding to heterodimers of Ube2N/Uev1a via its RING domain and recruiting additional E2 conjugating enzymes which allow for K48 linked poly-ubiquitylation to occur as well as K63 linked polyubiquitylation (Plans *et al.*, 2006).

References:

Araki T, Nagarajan R, Milbrandt J (2001) Identification of genes induced in peripheral nerve after injury: expression profiling and novel gene discovery. *J Biol Chem* **276**, 34131-34141.

Araki T, Milbrandt J (2003) ZNRF proteins constitute a family of presynaptic E3 ubiquitin ligases. *J Neurosci* **23**, 9385-9394.

Plans V, Scheper J, Soler M, Loukili N, Okano Y, Thomson TM. (2006) The RING finger protein RNF8 recruits UBC13 for lysine 63-based self polyubiquitylation. JCell Biochem 97, 572-82.

Physical Characteristics

Species: human

Source: *E. coli*

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: ~50 kDa

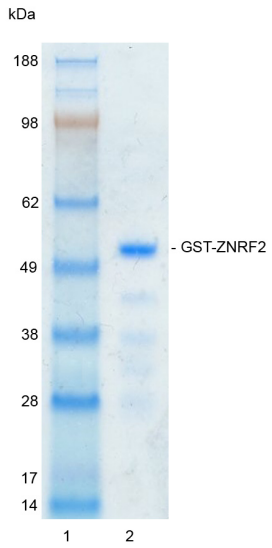
Purity: >67% by InstantBlue™ SDS-PAGE

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

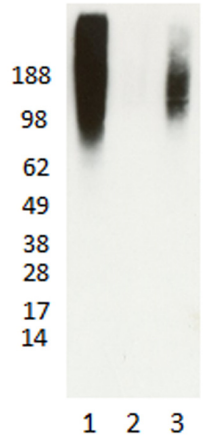
Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-ZNRF2



Protein Identification:
Confirmed by mass spectrometry.



E3 ligase assay: The ubiquitin conjugating activity of GST-ZNRF2 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-UBE2D3 (UbcH5c) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of GST-ZNRF2 for 30 minutes at 30˚C in the presence of ubiquitin, His-UBE1, His-UBE2D3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-ZNRF2 (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-ZNRF2.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
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

ZNRF2 [GST-tagged]

E3 Ligase

Alternate Names: Zinc and ring finger protein 2

Cat. No.	**63-0041-025**	Quantity:	25 µg
Lot. No.	**30217**	Storage:	-70˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS | **CERTIFICATE OF ANALYSIS Page 2 of 2**

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHN
MLGGCPKERAEISMLEGAVLDIRYGVSRI
AYSKDFETLKVDFLSKLPEMLKMFEDRLCH
KTYLNGDHVTHPDFMLYDALDVVLYM
DPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKS
DLEVLFQGPLGS***M***GAKQSGPAAANGR
TRAYSGSDLPSSSSGGANGTAGGGGGA
RAAAAGRFPAQVPSAHQPSASGGAAAAAAA
PAAPAAPRSRSLGGAVGSVASGA
RAAQSPFSIPNSSSGPYGSQDSVHSSPEDG
GGGRDRPVGGSPGGPRLVIGSLPAHLSPHMF
GGFKCPVCSKFVSSDEMDLHLVMCLTKPRI
TYNEDVLSKDAGECAICLEELQQGDTIARLP
CLCIYHKGCIDEWFEVNRSCPEHPSD

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
ZNRF2 (regular text): Start ***bold italics*** (amino acid residues 1-242)
Accession number: Q8NHG8

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
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