

ZNRF1 [GST-tagged]

E3 Ligase

Alternate Names: Zinc and ring finger protein 1

Cat. No. 63-0040-025

Lot. No. 30216

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). Zinc and Ring Finger protein 1 (ZNRF1) 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). ZNRF1 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 ZNRF1 and mutation of the Zinc finger Ring finger domain of ZNRF1 showed that it was required for the E3 ligase activity of ZNRF1 (Araki and Milbrandt 2003). Over expression of ZNRF1 – which has been implicated in neuronal degeneration – has been shown to increase neurite outgrowth (Yoshida *et al.*, 2009). ZNRF1 has also been shown to cause the degradation of the protein kinase AKT thus preventing the phosphorylation of glycogen synthase kinase-3β thus inactivating it in axons leading to Wallerian Degeneration (Wakatsuki *et al.*, 2011).

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.

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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: >55% by InstantBlue™ SDS-PAGE

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

Protein Sequence: Please see page 2

Quality Assurance

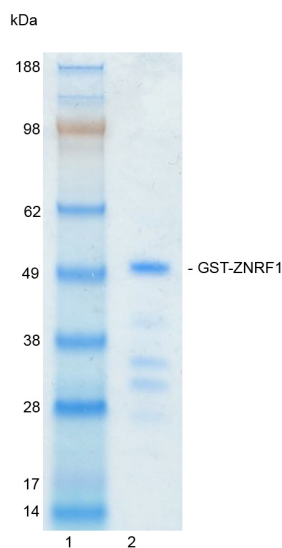
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

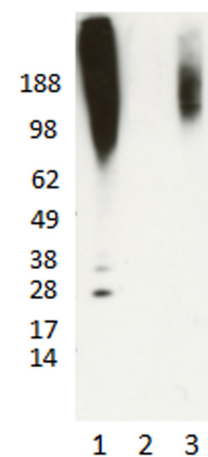
Lane 1: MW markers

Lane 2: 1 µg GST-ZNRF1



Protein Identification:

Confirmed by mass spectrometry.



E3 ligase assay: The ubiquitin conjugating activity of GST-ZNRF1 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-ZNRF1 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-ZNRF1 (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-ZNRF1.



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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

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

Wakatsuki S, Saitoh F, Araki T (2011) ZNRF1 promotes Wallerian degeneration by degrading AKT to induce GSK3B-dependent CRMP2 phosphorylation. *Nat Cell Biol* **12**, 1415-23.

Yoshida K, Watanabe M, Hatakeyama S. (2009) ZNRF1 interacts with tubulin and regulates cell morphogenesis *Biochem Biophys Res Commun* **389**, 506-11.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPPEMLKMFEDRLCHK
TYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAI PQIDKYLKSSKY
IAWPLQGWQATFGGGDHPKSDLEVL FQG
PLGSMGGKQSTAARSRGPFPVSTDDSAVP
PPGGAPHFHGYRTGGGAMGLRSRSVSSVAG
MGMDPSTAGGVFFGLYTPASRGTGDSER
APGGGSASDSTYAHNGYQETGGGHHRDG
MLYLGSRASLADALPLHIAPRWFSSHS
GFKCPICSKSVASDEMEMHFIMCLSKPRL
SYNDDVLTKDAGECVICLELLQGD TIARLP
CLCIYHKSCIDSWFEVNRSCPEHPAD

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (**LEVLFQ▼GP**)
ZNRF1 (regular text): Start **bold italics** (amino acid residues 1-227)
Accession number: Q8ND25



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