UBE2H (UbcH2) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: 6.3.2.19, E2-20K, UBC8, UbcH, UbcH2, Ubiquitin-conjugating enzyme E2H

Cat. No. 62-0078-020

Lot. No. 1829 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 regulated and targeted proteasomal 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). UBE2H is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Kaiser et al. (1994). Human UBE2H shares 54% identity with its yeast homoloque (UbcH8) and full length forms of both the human and yeast enzymes showed similar enzymatic activities in vitro by catalyzing the ubiquitylation of histones (Kaiser et al., 1995). In skeletal muscle whole cell extracts TNFα up-regulates and increases the conjugating activity of UBE2H in vitro via binding of NFkB to the promoter region of the gene (Li et al., 2003). UBE2H has been mapped to a region on chromosome 7 (Hayashida et al., 2000) and the gene has been identified as a candidate for involvement in an autistic disorder with neurodevelopmental complications. Single strand conformation analysis demonstrated a significant association between a polymorphism in the UBE2H gene suggesting it could be one of the 7qsusceptibility loci for Autistic Disorder (Vourc'h et al., 2003). An association has also been made between UBE2H and the motor neuron disorder amyo-

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Physical Characteristics

20 µg

Species: human

Quantity:

Source: E. coli expression

Quantity: 20 µg

Concentration: 1 mg/ml

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

Molecular Weight: ~23 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

aliquot as required

Protein Sequence:

 $\textbf{M} \; \textbf{G} \; \textbf{S} \; \textbf{S} \; \textbf{H} \; \textbf{H} \; \textbf{H} \; \textbf{H} \; \textbf{H} \; \textbf{H} \; \textbf{S} \; \textbf{S} \; \textbf{G} \; \texttt{L} \; \texttt{E} \; \texttt{V} \; \texttt{L} \; \texttt{F} \; \texttt{Q} \; \texttt{G}$ PGSMSSPSPGKRRMDTDV VKLIESKHEVTILGGLNEFVVK FYGPQGTPYEGGVWKVRVDLPD KYPFKSPSIGFMNKIFHPNIDEAS GTVCLDVINQTWTALYDLTNIFES FLPQLLAYPNPIDPLNGDAAAM YLHRPEEYKQKIKEYIQKYATEE ALKEQEEGTGDSSSESSMSDF SEDEAODMEL

1-183)

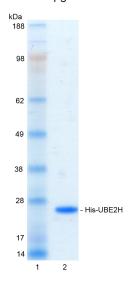
Tag (bold text): N-terminal His Protease cleavage site: PreScission™ (<u>LEVLFQ▼GP</u>) UBE2H (regular text): Start bold italics (amino acid residues

Accession number: NP 003335

Quality Assurance

Purity:

4-12% gradient SDS-PAGE InstantBlue™ staining Lane 1: MW markers Lane 2: 1 µg His-UBE2H



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of His-UBE2H was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2H E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2H enzymes in the presence of ubiquitin and ATP at 30°C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the ubiquitin/His-UBE2H thioester bond to the reducing agent DTT was confirmed.



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Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

trophic lateral sclerosis (ALS) where single strand conformation polymorphism (SSCP) analysis identified a known and sporadic polymorphism in the gene (Martin *et al.*, 2008).

References:

Hayashida S, Yamasaki K, Asada Y, Soeda E, Niikawa N, Kishino T (2000) Construction of a physical and transcript map flanking the imprinted MEST/PEG1 region at 7q32. *Genomics* 66, 221.5

Kaiser P, Mandl S, Schweiger M, Schneider R (1995) Characterization of functionally independent domains in the human ubiquitin conjugating enzyme UbcH2. FEBS Lett 377, 193-6.

Kaiser P, Seufert W, Hofferer L, Kofler B, Sachsenmaier C, Herzog H, Jentsch S, Schweiger M, Schneider R (1994) A human ubiquitin-conjugating enzyme homologous to yeast UBC8. *J Biol Chem* **269**, 8797-802.

Li YP, Lecker SH, Chen Y, Waddell ID, Goldberg AL, Reid MB (2003) TNF-alpha increases ubiquitin-conjugating activity in skeletal muscle by up-regulating UbcH2/E220k. FASEB J 17, 1048-57.

Martin I, Vourc'h P, et al. (2009) Association study of the ubiquitin conjugating enzyme gene UBE2H in sporadic ALS. *Amyotroph Lateral Scler* **10**, 432-5.

Vourc'h P, Martin I, Bonnet-Brilhault F, Marouillat S, Barthelemy C, Pierre Muh J, Andres C (2003) Mutation screening and association study of the UBE2H gene on chromosome 7q32 in autistic disorder. *Psychiatr Genet* 13, 221-5.



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