

# UBE2H (UbcH2) [GST-tagged]

## E2 – Ubiquitin Conjugating Enzyme

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

**Cat. No.** 62-0031-020  
**Lot. No.** 1396

**Quantity:** 20 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 regulated and targeted proteosomal 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 the yeast homologue (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 TNF $\alpha$  up-regulates and increases the conjugating activity of UBE2H *in vitro* via binding of NF- $\kappa$ B 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 7q-susceptibility loci for Autistic Disorder (Vourc'h *et al.*, 2003). An association has also been made between UBE2H and the motor neuron disorder amyotrophic 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.

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### Physical Characteristics

**Species:** human

**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:** ~47 kDa

**Purity:** >98% by InstantBlue™ SDS-PAGE

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

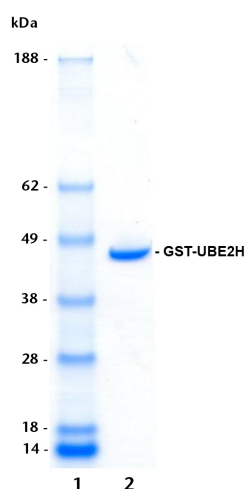
### Protein Sequence:

MSPILGYWKIKGLVQPTRLLEYLEEKYEEH  
LYERDEGDKWRNKKFELGLEFPNLPYYIDG  
VKLTQSMARIYIADKHNMLGGCPKER  
AEISMLEGAVLDIRYGVSRAYSKDFETLKVD  
FLSKLPEMLKMFEDRLCHKTYLNGDHTVHP  
DFMLYDALDVVLYMDPMCLDAFPKLVCFK  
KRIEAIQIDKYLKSSKYIAWPLQGWQAT  
FGGGDHPPKSDLEVLFGQPLGSSPSPG  
KRRMDTDVVKLIKESKHEVTILGGLNEFVVK  
FYGPQGTPEYEGGVWVVRVLDLPDKYFPKSPSIG  
FMNKIFHPNIDEASGTVCLDVINQWTALY  
DLTNIFESFLPQLLAYPNPIDPLNGDAAAM  
YLHRPEEYKQKIKEYIQKYATEEALKEQEEGT  
GDSSSESSMSDFSEDAQDMEL

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)  
Protease cleavage site: PreScission™ (LEVL**FGQ**GP)  
UBE2H (regular text): Start **bold italics** (amino acid residues 2-183)  
Accession number: NP\_003335

### Quality Assurance

**Purity:**  
4-12% gradient SDS-PAGE  
InstantBlue™ staining  
lane 1: MW markers  
lane 2: 1 µg GST-UBE2H



### Protein Identification:

Confirmed by mass spectrometry.

### E2-Ubiquitin Thioester Loading Assay:

The activity of GST-UBE2H was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2H E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2H enzymes in the presence of ubiquitin and ATP at 30°C was compared at two time points, T<sub>0</sub> and T<sub>10</sub> minutes. Sensitivity of the ubiquitin/GST-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

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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, Mahe M, Thepault RA, Antar C, Vedrine S, Praline J, Camu W, Andres CR, Corcia P (2008) Association study of the ubiquitin conjugating enzyme gene UBE2H in sporadic ALS. *Amyotroph Lateral Scler*, 1-4.

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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