

Product name(s):	[C ⁸⁵ A]Ubch5b (His ₆ -tagged)				
Catalogue number:	UW9065	Batch number:	Z03720	Expiry date:	12 months from receipt
Product information:					
<p>A number of E2s in <i>Saccharomyces cerevisiae</i> and their homologues have been identified. One such family of E2s is the UBC4/5, characterised as essential for the degradation of short-lived, regulatory and abnormal proteins¹. Protein levels of <i>S. cerevisiae</i> UbC4/5 are up-regulated in response to stress, and their loss results in severe effects on cellular functions².</p> <p>A human gene product that is 79% identical to <i>S. cerevisiae</i> UBC4/5 in amino-acid sequence was identified as Ubch5a³. In addition, two other human members of this highly conserved E2 class were also cloned and designated as Ubch5b and Ubch5c⁴, having 88% and 89% identity to Ubch5a, respectively.</p> <p>Members of the Ubch5a/b/c are the most active class of E2s in cell extracts. The importance of this enzyme class is underscored by the critical role of UBC4/5 in <i>S. cerevisiae</i>. Ubch5a stimulates the conjugation of ubiquitin to the tumour-repressor p53 in the presence of E6-AP and E6³. Moreover, Ubch5 family is implicated in <i>c-fos</i> recognition, the modulation of which is controlled by the ubiquitin-proteasome pathway⁵. Ubch5b and Ubch5c are associated with the signal-induced conjugation and subsequent degradation of IκBα in the presence of the SCF complexes (Skp/Cul/F-Box)⁶. Ubch5c also catalyses the ubiquitination leading to the processing of p105 precursor to form p50, a subunit of the heterodimeric transcription factor NF-κB⁷. The range and diversity of substrates and E3s with which this class of E2 enzymes interact, suggest their complex roles in cellular functions require to be studied further.</p>					
Application data:					
<p>The His₆-tagged fusion proteins of Ubch5a, Ubch5b and Ubch5c all charge and support ubiquitinylation <i>in vitro</i>. Unlike their GST-tagged counterparts, the His₆-tagged Ubch5 family members all appear to form thiol ester conjugates with ubiquitin at a similar rate under similar conditions. The [C⁸⁵A] mutation completely abolishes the ability of the enzyme to form a thiol ester and thereby acts as an excellent negative control.</p> <p>Purity determined to be >95% by SDS-PAGE.</p>					
Storage and use:					
<p>Material is supplied at a concentration of 1.0mg/mL in 20mM Tris-HCl, pH7.5, containing 0.5mM DTT. For long term storage store at -80C. Enzyme is stable to multiple freeze/thaw cycles.</p>					
References:					
<ol style="list-style-type: none"> Seufert, W. and Jentsch, S. Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of short-lived and abnormal proteins. <i>EMBO J.</i>, 9, 543-50 (1990) Jentsch, S. The ubiquitin-conjugation system. <i>Annu. Rev. Genet.</i>, 22, 179-207 (1992) Scheffner, M. Identification of a human ubiquitin-conjugating enzyme that mediates the E6-AP-dependent ubiquitination of p53. <i>Proc. Natl. Acad. Sci. USA</i>, 91, 8797-8801 (1994) Jensen, J.P., Bates, P.W., Yang, M., Vierstra, R.D. and Weissman, A.M. Identification of a family of closely related human ubiquitin conjugating enzymes. <i>J. Biol. Chem.</i>, 270, 30408-14 (1995) Stancovski, I., Gonen, H., Orian, A., Schwartz, A.L. and Ciechanover, A. Degradation of the proto-oncogene c-Fos by the ubiquitin proteolytic system <i>in vivo</i> and <i>in vitro</i>: identification and characterisation of the conjugating enzymes. <i>Mol. Cell. Biol.</i>, 15, 7106-16 (1995) Gonen, H., Bercovich, B., Orian, A., Carrano, A., Takizawa, C., Yamanaka, K., Pagano, M., Iwai, K. and Ciechanover, A. Identification of the ubiquitin carrier proteins, E2s, involved in signal-induced conjugation and subsequent degradation of IκBα. <i>J. Biol. Chem.</i>, 274, 14823-30 (1999) Coux, O. and Goldberg, A.L. Enzymes catalyzing ubiquitination and proteolytic processing of the p105 precursor of nuclear factor κB1. <i>J. Biol. Chem.</i>, 273, 8820-8 (1998) 					