UBE2C (UbcH10) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: Cyclin selective ubiquitin carrier protein, EC 6.3.2.19, Mitotic specific ubiquitin conjugating enzyme, UbcH10, Ubiquitin carrier protein E2 C, Ubiquitin conjugating enzyme UbcH10

Cat. No. **62-0006-020**
Lot. No. **1386**

Quantity: 20 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

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). UBE2C is a member of the E2 ubiquitin-conjugating enzyme family and cloning of the human gene was first described by Townsley *et al.* (1997). UBE2C shares 85% and 61% homology with frog and clam sequences respectively, and has a 30 amino acid N-terminal extension. UBE2C binds APC11 and APC2 resulting in their autoubiquitylation and proteosomal degradation, a switch in the APC complex which causes sister chromatid separation and exit from mitosis (Rape and Kirschner, 2004; Tang *et al.*, 2001). UBE2C has also been identified as a molecular marker useful in non small cell lung carcinoma (NSCLC) prognosis (Kadara *et al.*, 2009).

References:

Kadara H, Lacroix L, Behrens C, Solis L, Gu X, Lee JJ, Tahara E, Lotan D, Hong WK, Wistuba II, Lotan R (2009) Identification of gene signatures and molecular markers for human lung cancer prognosis using an in vitro lung carcinogenesis system. *Cancer Prev Res (Phila Pa)* **2**, 702-11.

Rape M, Kirschner MW (2004) Autonomous regulation of the anaphase-promoting complex couples mitosis to S-phase entry. *Nature* **432**, 588-95.

Tang Z, Li B, Bharadwaj R, Zhu H, Ozkan E, Hakala K, Deisenhofer J, Yu H (2001) APC2 Cullin protein and APC11 RING protein comprise the minimal ubiquitin ligase module of the anaphase-promoting complex. *Mol Biol Cell* **12**, 3839-51.

Townsley FM, Aristarkhov A, Beck S, Hershko A, Ruderman JV (1997) Dominant-negative cyclin-selective ubiquitin carrier protein E2-C/UbcH10 blocks cells in metaphase. *Proc Natl Acad Sci USA* **94**, 2362-7.

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: ~46 kDa

Purity: >90% by InstantBlue™ SDS-PAGE

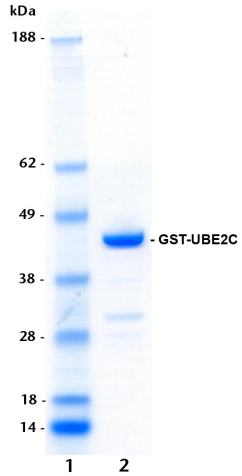
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH LYERDEGDKWRNKKFELGLEFPNLPYYIDGD VKLTQSMAIIRYIADKHNMLGGCPKER AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP DFMLYDALDVVLYMDPMCLDAFP KLVCFKKRIEAIPQIDKYLKSSKYIAWPLQG WQATFGGGDHPPKSDLEVLFQGPLGS ***M***ASQNRDPAATSVAAARKGAEPSGGAARG PVGKRLQQELMTLMMSGDKGISAFPESDN LFKWVGTIHGAAGTVYEDLRYKLSLEFPSGY PYNAPTVKFLTPCYHPNVDTQGNICLDILKE KWSALYDVRTILLSIQSLLGEPNIDSPLNTHAAEL WKNPTAFKKYLQETYSKQVTSQEP

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2C (regular text): Start ***bold italics*** (amino acid residues 1-179)
Accession number: NP_008950.1

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg GST-UBE2C



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2C was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2C E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2C 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/GST-UBE2C thioester bond to the reducing agent DTT was confirmed.

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Lot-specific COA version tracker: v1.0.0