Lot # XXXXX

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MATERIAL DATA SHEET

UbcH5c/UBE2D3-Ubiquitin Charged, *human recombinant*
Cat. # E2-802

Ubiquitin-conjugating Enzyme H5c (UbcH5c), also known as Ubiquitin-conjugating Enzyme E2D 3 (UBE2D3), is a member of the yeast Ubc4/5 family of Ubiquitin-conjugating (E2) enzymes. Human UbcH5c/UBE2D3 has a predicted molecular weight of 17 kDa and shares 88% and 89% amino acid sequence identity with the related family members, UbcH5a and UbcH5b, respectively. In combination with Ubiquitin ligases (E3s) such as CHIP, UbcH5c/UBE2D3 mediates the ubiquitination and subsequent degradation of several regulatory proteins. For instance, UbcH5c/UBE2D3 is involved in the polyubiquitination and proteasome-mediated degradation of the Nuclear Factor kappaB (NF-κB) inhibitor, IκB-α, and is implicated in NF-κB-dependent inflammation. UbcH5c/UBE2D3 also mediates the ubiquitination of Histone H2A and PCNA, suggesting that it functions during transcriptional regulation, DNA replication, and DNA damage responses.

This product is an enzymatically generated UbcH5c/UBE2D3-Ubiquitin thioester complex that has been highly purified to remove E1 Ubiquitin Activating enzyme, uncharged UbcH5c/UBE2D3, free Ubiquitin, and Mg^{2+}-ATP. The product provides a convenient starting material for use in single-turnover "Ubiquitin Discharge Assays," eliminating the need to either inhibit the E1 Ubiquitin Activating enzyme with potentially confounding chemical treatments or remove ATP via enzyme additions.

Product Information	
Quantity:	100 µg
Stock:	X mg/ml (X µM) in 50 mM HEPES pH 7.5, 50 mM NaCl, 1 mM TCEP
MW:	25 kDa (17 kDa UBE2D3, 8.6 kDa Ubiquitin)
Purity:	> 95% by SDS-PAGE under non-reducing conditions and visualized by Colloidal Coomassie Blue stain

Use & Storage	
Use:	Pre-charged UBE2D3 may be added directly to *in vitro* reactions containing E3 Ubiquitin ligases and ligase substrates (if applicable)—no ATP, E1 enzyme, or extra Ubiquitin are required. Reaction conditions will need to be optimized for each specific application. Note: Reducing agents including dithiothreitol (DTT) or mercaptoethanol (βME) may cause unintended thiolytic release of Ubiquitin from the complex—care must be taken if these compounds are present in buffers. We suggest using a thioester friendly reductant such as TCEP if possible.
Storage:	Store at -80°C. Avoid multiple freeze-thaw cycles. Avoid DTT, βME, and other thiol-containing compounds in buffers.

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Literature

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