Lot # XXXXX

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MATERIAL DATA SHEET

UbcH5a/UBE2D1-Ubiquitin Charged, *human recombinant*
Cat. # E2-800

Ubiquitin-conjugating Enzyme H5a (UbcH5a), also known as Ubiquitin-conjugating Enzyme E2D 1 (UBE2D1), is a ubiquitously expressed protein that is related to Stimulator of Iron Transport (SFT). Human UbcH5a/UBE2D1 has a predicted molecular weight of 17 kDa and shares 89% and 88% amino acid (aa) sequence identity with the related family members UbcH5b and UbcH5c, respectively. Human UbcH5a/UBE2D1 shares 100% aa sequence identity with the mouse and rat orthologs. UbcH5a/UBE2D1 has a conserved E2 catalytic core domain that contains an active site cysteine residue, and it interacts with a variety of HECT and RING finger Ubiquitin ligases (E3) to mediate the ubiquitination of c-Fos, RIP1, HIF-1, and other targets. Pathologically, UbcH5a/UBE2D1 is implicated in protein degradation during cancer and immune responses.

This product is an enzymatically generated UbcH5a/UBE2D1-Ubiquitin thioester complex that has been highly purified to remove E1 Ubiquitin Activating enzyme, uncharged UbcH5a/UBE2D1, 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 UBE2D1, 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 UBE2D1 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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Rev: 12/12/2014

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