Lot # XXXXX

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MATERIAL DATA SHEET

UbcH5b/UBE2D2-Ubiquitin Charged, *human recombinant*
Cat. # E2-801

Ubiquitin-conjugating Enzyme H5b (UbcH5b), also known as Ubiquitin-conjugating Enzyme E2D 2 (UBE2D2), is a widely expressed member of the Ubiquitin-conjugating (E2) enzyme family. The protein has a predicted molecular weight of 16.5 kDa. UbcH5b/UBE2D2 localizes to both the nucleus and the cytoplasm. The human protein shares 100% and 92% amino acid sequence identity with the mouse and rat orthologs, respectively. This enzyme has an E2 catalytic core domain that contains an active site cysteine residue that is required for the formation of a thioester bond with Ubiquitin. UbcH5b/UBE2D2 is capable of mediating the formation of Ubiquitin chains linked through Lys11, Lys48, or Lys63. Working with the SCF(Fbxw2) and MDM2/HDM2 Ubiquitin ligases (E3s), UbcH5b/UBE2D2 mediates the ubiquitination and degradation of the transcription factors GCM1 and p53, respectively. Along with UBE2N/Ubc13, UbcH5b/UBE2D2 may have a role in the endocytosis and endolysosomal degradation of MHC class I molecules. Non-proteolytic ubiquitination of TRIM5-α by UbcH5b/UBE2D2 has been reported to block HIV reverse transcription. Pathologically, UBE2D family members may be critical targets of cadmium during cadmium-induced renal toxicity. Additionally, overexpression of UbcH5b/UBE2D2 has been linked to inflammatory bowel disease.

This product is an enzymatically generated UbcH5b/UBE2D2-Ubiquitin thioester complex that has been highly purified to remove E1 Ubiquitin Activating enzyme, uncharged UbcH5b/UBE2D2, 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 UBE2D2, 8.6 kDa Ubiquitin)
Purity:	> 95% by SDS-PAGE under non-reducing conditions and visualized by Colloidal Coomassie Blue stain

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Use & Storage	
Use:	Pre-charged UBE2D2 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.

Literature

References:

Das, R. *et al.* (2013) EMBO J. **32**: 2504
Koyano, F. *et al.* (2014) Nature **510**: 162
Liew, C.W. *et al.* (2010) Biochem. J. **431**: 23
Petroski, M.D. & Deshaies, R.J. (2005) Cell **123**: 1107
Saha, A. *et al.* (2011) Mol. Cell **42**: 75

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