
MATERIAL DATA SHEET

Recombinant Human UbcH5a/UBE2D1 Ubiquitin Charged**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 cFos, RIP1, HIF1 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 purified to remove E1 Ubiquitin Activating enzyme, uncharged UbcH5a/UBE2D1, free Ubiquitin, and Mg²⁺-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
MW:	25 kDa (17 kDa UBE2D1, 8.6 kDa Ubiquitin)
Source:	<i>E. coli</i> -derived Accession # P51668, P0CG47
Stock:	Approximately 0.43 mg/ml (25 µM) with respect to UBE2D1. Formulated in 50 mM HEPES pH 7.5, 50 mM NaCl, 1 mM TCEP
Purity:	>95%, by SDS-PAGE under 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 (β ME) 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: Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

Literature

References:

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

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