

ProductInformation

UBIQUITIN CONJUGATING ENZYME FRACTIONS

Mammalian

Product Number **U8382**

Product Description

Ubiquitin Conjugating Enzyme Fractions, isolated from rabbit, represent the full complement of purified conjugation enzymes (E1, E2s, and E3s) that are found in mammalian Fraction II. These conjugation fractions also contain ubiquitin c-terminal hydrolases (UCHs). Ubiquitin Conjugating Enzyme Fractions do not contain 20S or 26S proteasome protein degradation activity.

Degradation of short-lived, key regulatory proteins by the ubiquitin-proteasome pathway plays key roles in a number of cellular processes. A number of proteins are degraded by this system including: cyclins, cyclin-dependent kinases^{1,2} and their inhibitors, tumor suppressors, oncoproteins, and transcriptional activators and their inhibitors. Two discrete steps are involved in the ubiquitin-mediated degradation of proteins: signaling by covalent conjugation of multiple ubiquitin moieties and degradation of the tagged substrate. Conjugation occurs by a three-step mechanism involving three different enzymes that act sequentially: E1, E2, and E3. Ubiquitin-activating enzyme (E1) catalyzes the activation of ubiquitin then E2 (ubiquitin-conjugating enzyme) transfers activated ubiquitin to E3, which is bound to substrate. E3 catalyzes the polyubiquitination of the targeted protein. The polyubiquitin tagged protein is then degraded by the 26S proteasome in an ATP-dependent process and free ubiquitin is released.³⁻⁵

Reagent

Ubiquitin Conjugating Enzyme Fractions are supplied as two enzyme fraction solutions each in 50 mM HEPES, pH 7.6 with 0.1 mM DTT. Fraction 1 (Product No. U 1383) contains primarily the majority of E1 and E2 enzymes, while Fraction 2 (Product No. U 1258) primarily contains the E3 ligases. Due to the method of preparation there is some overlap of fraction contents.

Precautions and Disclaimer

For laboratory use only. Not for drug, household or other uses. Please consult the Material Safety Data Sheet for handling recommendations before working with this material.

Storage/Stability

Ubiquitin Conjugating Enzyme Fractions should be stored in aliquots at -70°C . Avoid multiple freeze thaw cycles. Do not store in a frost-free freezer.

Product Profile

The enzymes have been tested and shown to work with typical ^{125}I -labeled substrate proteins such as lysozyme and β -lactoglobulin. The addition of ubiquitin aldehyde (U 1507) is recommended for the inhibition of UCHs and to improve overall conjugate yield.

References

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2. Yew, P.R., Ubiquitin-mediated proteolysis of vertebrate G1 and S-phase regulators. *J. Cell Physiol.*, **187**, 1-10 (2001).
3. Tanaka, K., et al., The ligation systems for ubiquitin and ubiquitin-like proteins. *Mol. Cells*, **8**, 503-512 (1998).
4. Myung, J., et al., The ubiquitin-proteasome pathway and proteasome inhibitors. *Med. Res. Rev.*, **21**, 245-273 (2001).
5. Benaroudj, N., et al., The unfolding of substrates and ubiquitin-independent protein degradation by proteasomes. *Biochimie*, **83**, 311-318 (2001).

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