His6-USP16, human recombinant
Cat. # E-612

USP16 is a deubiquitinating enzyme (DUB) of the C19 peptidase family, USP16 subfamily. Human USP16 has a predicted molecular weight of 94 kDa and is 83% identical to both mouse and rat orthologues. USP16 plays significant roles in cell-cycle progression and Hox gene expression, and USP16 knockdown in HeLa cells results in defects in the mitotic phase of the cell cycle. Studies reveal that histone H2A deubiquitination by USP16 is a prerequisite for phosphorylation of Serine 10 of histone H3 and chromosome segregation during mitosis. USP16 specifically deubiquitinates histone H2A, but not H2B, both in vitro and in vivo. PLK1 serine/threonine kinase is another reported target of USP16. Finally, a chromosomal inversion involving USP16 and RUNX1/AML1 is a causative event in the development of chronic myelomonocytic leukemia (CMML). This recombinant protein contains an N-terminal 6-His tag.

Product Information

| Quantity: | 25 μg |
| Stock: | X mg/ml (X μM) in 50 mM HEPES pH 7.5, 100 mM NaCl, 10% (v/v) Glycerol, 1 mM TCEP |
| MW: | 94 kDa |
| Purity: | > 85% by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie Blue stain |

Use & Storage

Use:
Recombinant Human USP16 is a Ubiquitin-specific deconjugating enzyme. Reaction conditions will need to be optimized for each specific application. USP16 does not efficiently utilize Ubiquitin-AMC (U-550), Ubiquitin-Rhodamine 110 (U-555), nor unanchored poly-Ubiquitin chains as substrates in vitro. USP16 cleaves Ubiquitin from recombinant, designer nucleosomes (see Sample Data).

Storage:
Store at -80°C. Avoid multiple freeze/thaw cycles.

Literature

References:
USP16 deubiquitinates mono- and di-ubiquitinated histone H2A in nucleosomes.

Ubiquitinated Designer Nucleosomes (UDN’s), co-developed by Boston Biochem and EpiCypher™, were used as substrates in *in vitro* deubiquitinase (DUB) assays.

Left Panel: UDN-H2A containing histone H3.1. Right Panel: UDN-H2A containing histone H3.3. 20 µl reactions containing 500 nM UDN and either 100 nM USP30, USP16 or no deubiquitinating enzyme were incubated at 37°C for 45 minutes and then quenched with SDS-PAGE buffer. Samples were resolved on 10-20% gradient PAGE gels and visualized by Collodial Coomassie Blue stain. The UDN-H2A substrates consist of a population of nucleosomes that are ubiquitinated at one or two residues on histone H2A. Using these conditions, USP16 removed Ubiquitin from both histone H2A sites within the context of the nucleosome substrates. USP30 had trace activity using the UDN-H2A substrates.

Substrate details

Ubiquitinated Nucleosomes are generated at Boston Biochem using recombinant designer nucleosomes supplied by EpiCypher. The H3.1-based substrate is built on EpiCypher catalog number 16-0009, containing the following histones: H2A (UniProt P04908), H2B (UniProt O60814), H3.1 (UniProt P68431) and H4 (UniProt P62805). The nucleosome DNA consists of a 147 base pair “601” positioning sequence DNA.

The H3.3-based substrate is similarly generated using EpiCypher catalog number 16-0012, containing H2A (UniProt P04908), H2B (UniProt O60814), H3.1 (UniProt P84243) and H4 (UniProt P62805).