Biotinylated K48-Linked Tetra-Ubiquitin (Phosphorylated)

Cat. # SI4804BP

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Background

Polyubiquitination of target proteins through lysine 48 (K48) is the most thoroughly studied of the various ubiquitin chain linkages and was once considered the hallmark of ubiquitination. It is now clear that many, if not all, polyubiquitin chain topologies play distinct and important roles in regulating cellular processes. Nevertheless, K48-linked polyubiquitination remains the primary signal for proteasome-mediated degradation.

K48-linked tetra-ubiquitin chains are generated enzymatically using the E2-25K enzyme, which links wild-type ubiquitin molecules through lysine 48. The most distal ubiquitin in the chain contains an arginine substitution at position 48, preventing further elongation and limiting chain length. The chains are then enzymatically phosphorylated at the Ser65 residue. Once phosphorylated, these tetra-ubiquitin chains are biotinylated on a mutated cysteine introduced into one of the ubiquitin monomers, allowing the attachment of a single biotin molecule.

Application(s)

- Labeling proteins with biotinylated chains for selective separation using methods such as Western blotting, pull-downs (see website), ELISAs, etc.
- Visualizing biotinylated proteins using streptavidin or avidin detection systems
- Investigating phosphoubiquitin chain specificity and selectivity
- Studying the role of phosphoubiquitin chains in protein degradation pathways (e.g., proteasomal and autophagic degradation)
- Analyzing phosphoubiquitin-mediated signaling pathways and cellular responses
- Elucidating the structure and dynamics of phosphoubiquitin chains through structural studies
- Identifying modulators of phosphoubiquitin chain assembly and disassembly using screening assays

Product Specifications

Tag	Biotin
Purity	≥ 95% by HPLC-MS
Molecular Weight	34,842-35,082 Da (depending on the degree of phosphorylation)
Quantity	25 µg
Species	Human
Expression System	*E. Coli*
Physical State	Liquid
Buffer	50 mM Tris-HCl, pH 7.5, 150 mM NaCl
Solubility	> 1mg/mL
Stability & Storage	-80°C. Avoid repeated freeze/thaw cycles

References

1. Ohtake, F. Branched Ubiquitin Code: From Basic Biology to Targeted Protein Degradation. *J. Biochem.* **2022**, *171*, 361-366.
2. Swatek, K.N. & Komander, D. Ubiquitin Modifications. Cell Res. **2016**, *26*, 399-422.
3. Yau, R. & Rape, M. The increasing complexity of the ubiquitin code. Nature Cell. Bio. **2016**, 18, 579-586.