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# MATERIAL DATA SHEET

## Recombinant Human phospho-Tetra-Ubiquitin/Ub4 WT Chains (K63-linked, pS65)

## Cat. # UC-350

Linkage specific phosphorylated Poly-Ubiquitin chains may be used as a substrate for *in vitro* reactions with deubiquitinating enzymes ("DUB's") that cleave the peptide or isopeptide linkage between adjacent Ubiquitin molecules. Phosphorylated Poly-Ubiquitin chains can also be used to investigate mechanisms of binding and recognition between the chains and other proteins that contain Ubiquitin-Associated domains (UBAs), Ubiquitin-interacting motifs (UIMs), ZnF's and/or other Ubiquitin-sensing elements.

K63-linked Tetra-Ubiquitin chains phosphorylated at Serine 65 are manufactured using recombinant wild-type human recombinant Ubiquitin, linkage-specific enzymes, and PINK1 kinase. The use of purely enzymatic techniques avoids the potential for contaminating synthetic intermediates. The correctness of linkage and purity of each production lot is assessed using the Absolute Quantitation of Ubiquitin method (Ub-AQUA), an LCMS-based technique that provides extremely accurate information on the composition of Poly-Ubiquitin samples.

| Product Information | |
|---|---|
| **Quantity:** | 25 µg |
| **MW:** | 34 kDa |
| **Source:** | *E. coli*-derived<br>Accession # P0CG47 |
| **Stock:** | 1 mg/ml (29 µM) in sterile, deionized water |
| **Purity:** | >95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain. |

**Ub-AQUA analysis**:

K63: 99.04%

K11: 0.76%

K6 : 0.13%

All other linkages ≤ 0.04%

p-S65: 95.7%



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## Use & Storage

**Use:** Ubiquitin chains vary in length, linkage, and function. K63-linked, pS65 Tetra-Ubiquitin chains are ideal for investigating Ubiquitin-binding proteins and as substrates for Ubiquitin-specific isopeptidases. Reaction conditions will need to be optimized for each specific application. IMPORTANT: Heating this product in SDS-PAGE buffer or terminating reactions containing this product with heated SDS-PAGE buffer could lead to unexpected, high apparent molecular weight banding or smearing on gels that is not representative of product purity. For optimal results, we recommend incubation in SDS-PAGE buffer + DTT at ≤40 °C for 20 minutes prior to gel electrophoresis

**Storage:** **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 12 months from date of receipt, -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

## Literature

**References:**

1. Heo, J.M., Ordureau A., *et al.* (2015) Mol. Cell **60(1)**: 7-20
2. Kirkpatrick D.S., *et al.* (2006) Nat Cell Biol. **8(7)**: 700-10
3. Ordureau, A., *et al.* (2014) Mol. Cell **56(3)**: 360–375
4. Ordureau, A., *et al.* (2015) Pro. Nat. Acad. of Sci. USA **112(21)**: 6637–6642
5. Phu L., *et al.* (2011) Mol Cell Proteomics **10(5)**: M110.003756

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