([K11-only]Ub)n-ubiquitinylated substrate

Polyubiquitin chains, linked through specific lysine residues, are useful tools for investigating the specificity and reactivity of deubiquitinylating enzymes (DUBs) and the recognition and interaction of polyubiquitin modified proteins with ubiquitin binding domain (UBDs) containing proteins.

Ordering Information

Order Online »

BML-UW0620-0025	25µg

Manuals, SDS & CofA

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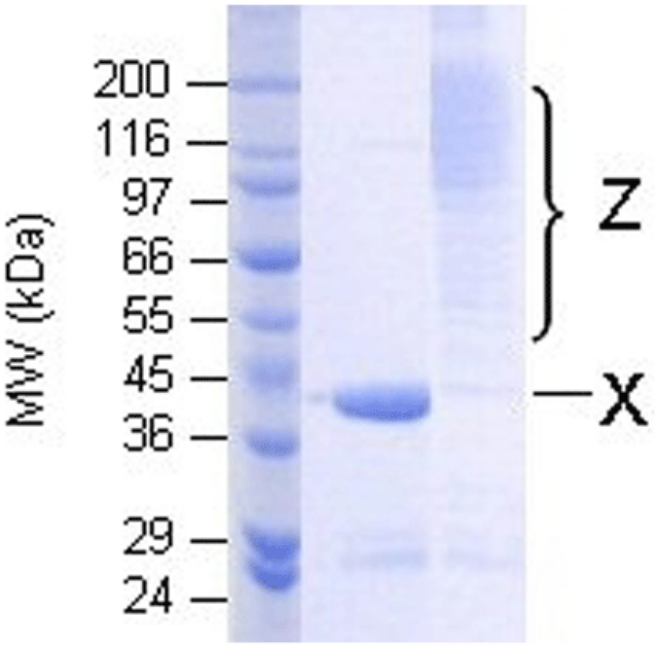


Figure 1: Coomassie stained SDS-PAGE gel showing ubiquitinylation of substrate protein (X) with (K^{11}-only) Ub to give (K^{11}-only)Ub_n-ubiquitinylated conjugates (Z; Prod. No. BML-UW0620).



Handling & Storage

Use/Stability	As indicated on product label or CoA when stored as recommended. Stable for at least 6 months after receipt when stored at -80°C.
Handling	Avoid freeze/thaw cycles. After opening, prepare aliquots and store at -80°C.
Short Term Storage	+4°C
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application Notes	**Uses:** 1. Deubiquitinylating enzyme substrates (general/linkage specific). 2. Profiling of DUB linkage-type preference or specificity in combination with other single lysine only polyubiquitinylated substrates (e.g. (K^6-only)Ub_n-ubiquitinylated substrate, Prod. No. BML-UW0615) 3. Investigation of polyubiquitin chain recognition by and interaction with ubiquitin binding proteins.
Formulation	Liquid. In 50mM TRIS, pH 7.5, containing 150mM NaCl and 1mM DTT.
MW	~50-250kDa
Purity	≥95% (SDS-PAGE)
Purity Detail	High molecular weight conjugates were separated from free ubiquitin by size exclusion chromatography.

Quality Control

SDS-PAGE: Multiple high molecular weight bands are observed for (K^{11}-only) Ub conjugate reactions (Fig. 1).
Immunoblotting: Detection of high molecular weight bands with the polyubiquitin-chain reactive monoclonal antibody FK2 (Prod. No. BML-PW8810) demonstrates their polyubiquitinylated, rather than mono- or multiubiquitinylated, status (Fig. 2).
Mass spectrometry: Conjugates were analyzed by mass spectrometry following tryptic digest. A signature peptide corresponding to the predicted branched ubiquitin fragments (H-TLTGK(G-G)TITLEVEPSDTIENVK-OH) was identified, confirming the polyubiquitinylated status of the conjugates and the nature of the linkage type.
DUB reactivity: The potential utility of (K^{11}-only)Ub_n-ubiquitinylated conjugates as DUB substrates was demonstrated by their deconjugation in the presence of the isolated catalytic domain of USP2 (Prod. No. BML-UW9850), a DUB of general and promiscuous activity that appears to exhibit no linkage preference (Fig. 3).

Source

Protein components produced in *E. coli*. Synthesized enzymatically *in vitro*. (K^{11}-only)Ub_n-ubiquitinylated substrate protein was produced using K^{11}-only mutant ubiquitin.

Technical Info / Product Notes

TECHNICAL NOTE: Under certain conditions these polyubiquitinylated substrates can bind to glutathione-derivatised matrices. This may have implications for their successful use in some applications, for example, if they are to be used in concert with GST-tagged proteins requiring subsequent affinity isolation with glutathione-based matrices. Such methods of detection or isolation should be avoided wherever possible in order to avoid complication in interpretation of results obtained.

UniProt ID

P0CG47 (UBB), P0CG48 (UBC), P62979 (RPS27A), P62987 (UBA52)

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