

**MATERIAL DATA SHEET****Tetra-Ubiquitin (K63-linked) Rhodamine 110****Cat. # UC-355**

Linkage specific 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. K63-linked tetra-Ubiquitin chains are manufactured using recombinant wild-type human Ubiquitin and linkage-specific enzymes. The use of purely enzymatic techniques avoids the potential for contaminating synthetic intermediates.

This fluorogenic substrate is intended for use with deubiquitinating enzymes that are unable to use mono-Ubiquitin-Rh110 (**U-555**) as a substrate, or enzymes with activity that is stimulated by poly-Ubiquitin chains. The substrate consists of a K63-linked tetra-Ubiquitin chain with a single rhodamine attached to the C-terminus of the proximal Ubiquitin.

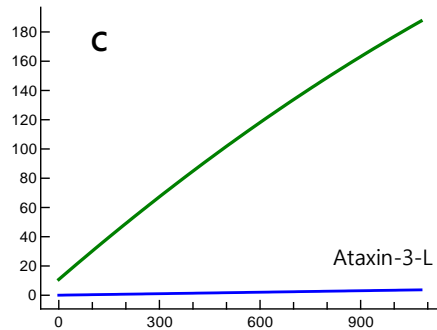
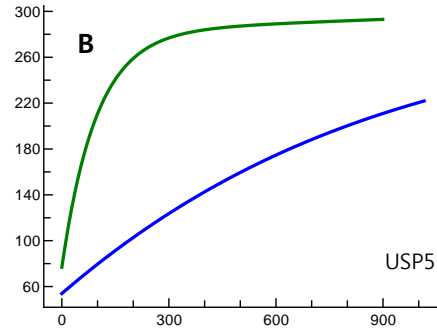
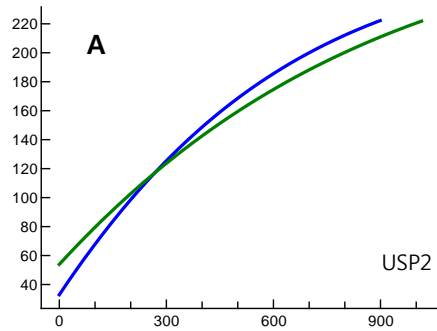
**Product Information**

<b>Quantity:</b>	50 µg
<b>Stock:</b>	X mg/ml (X µM) in 50 mM HEPES pH 7.0, 50 mM NaCl
<b>Purity:</b>	> 95% by SDS-PAGE under reducing conditions and visualized by both Colloidal Coomassie Blue stain and UV light
<b>MW:</b>	35 kDa

**Use & Storage**

<b>Use:</b>	K63-linked tetra-Ubiquitin Rhodamine 110 is ideal for use in assays requiring fluorescent detection of deubiquitinase activity. Optimal fluorescence at pH 8.0 is monitored with excitation and emission wavelengths of 485 nm and 535 nm, respectively. Reaction conditions will need to be optimized for each specific application. We recommend an initial concentration of 0.1-1 µM.
<b>Storage:</b>	Store at -20°C. Avoid multiple freeze/thaw cycles.

## Example Data



Three deubiquitinating enzymes using mono-Ubiquitin-Rhodamine 110 (cat # **U-555**) and K63-linked Tetra-Ubiquitin-Rhodamine110 (cat # **UC-355**) substrates. Continuous monitoring was done using a standard reaction buffer and  $\leq 10$  nM enzyme. Y-axis units are in RFU's, while X-axis units are reaction times in seconds. U-555 traces are in blue and UC-355 traces are in green. **Panel A:** USP2 catalytic core shows little preference for one or the other substrate. **Panel B:** USP5 exhibits substantially greater activity using UC-355. **Panel C:** Ataxin-3-like (UniProt Q9H3M9) hydrolyzes UC-355, but not U-555.

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840 Memorial Drive, Cambridge, MA 02139 Phone: 617-576-2210 FAX: 617-492-3565  
[www.bostonbiochem.com](http://www.bostonbiochem.com)

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