K63-linked Tetraubiquitin-Rhodamine 110

Cat. # SI233

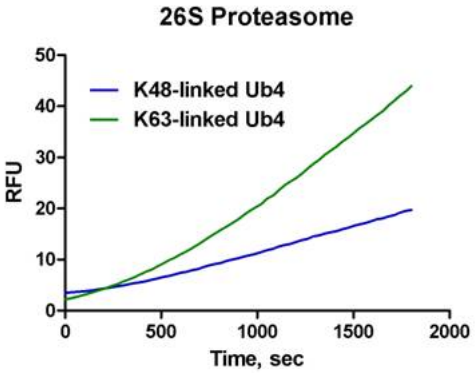
Background: K63-linked tetraubiquitin-rhodamine provides a sensitive, high throughput means for measuring Rpn11 activity towards K63-linked ubiquitin chains. Cleavage of the amide bond between the C-terminal glycine of ubiquitin and rhodamine results in an increase in rhodamine fluorescence at 535 nm (Exc. 485 nm).

Rpn11 (POH1), a JAMM-type isopeptidase, is one of three deubiquitinating enzymes associated with the 19S regulatory particle of the proteasome[1]. Substrate proteins are deubiquitinated by Rpn11 prior to unfolding and translocation into the 20S for degradation. In contrast to USP14 and UCH37 (UCH-L5), isopeptidases also associated with the 19S regulatory particle, Rpn11 deubiquitination promotes substrate degradation[2-7]. Therefore, modulators of Rpn11 could be effective means of controlling the function of the proteasome, a target for cancer, immune-related disorders, inflammation, neurodegeneration and other diseases. Historically, assays for Rpn11 have employed purified 26S proteasome (Cat. # PS026) as a source of enzyme and ubiquitinated protein as a substrate, methods requiring separation of cleaved from uncleaved substrate for determination of activity. Rpn11 activity can be distinguished from UCH37 and USP14 activity by a dependence on ATP, sensitivity to the chelator 1,10-phenanthroline (Cat. # SI9649) and insensitivity to the suicide DUB inhibitor ubiquitin-aldehyde (Cat. # SI250)[3].

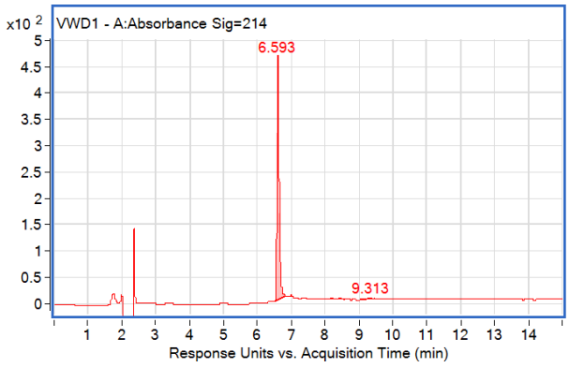
Application: Kinetic measurement of Rpn11 or other deubiquitinating activities. Screening for modulators of the Rpn11-mediated deubiquitinating activity of the 26S proteasome.

Product Information

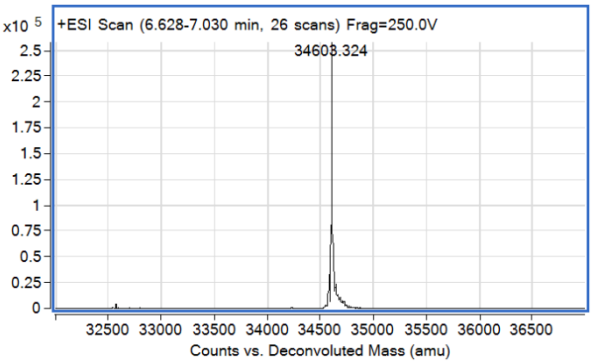
Purity:	≥ 90% by RP-HPLC
Molecular Weight:	34,602.7 Da (calculated)
Physical State:	Lyophilized solid. Resuspend in PBS or TBS (confirmed to be soluble at 50µM)
Quantity:	50µg
Ex/Em wavelengths:	Excitation: 485nm; Emission: 535nm
Storage:	-80°C. Avoid repeated freeze/thaw cycles



Isopeptidase activity of the 26S proteasome was measured with K48-linked tetraubiquitin-rhodamine 110 (Cat. # SI238; K48-linked Ub4; blue) and K63-linked tetraubiquitin-rhodamine 110 (Cat. # SI233; K63-linked Ub4; green) at 100nM as the increase in fluorescence at 535nm over time.



RP-HPLC



Deconvoluted mass spectrum

References

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2. Yao, T. and R.E. Cohen, *A cryptic protease couples deubiquitination and degradation by the proteasome.* Nature, 2002. **419**(6905): p. 403-7.
3. Verma, R., et al., *Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome.* Science, 2002. **298**(5593): p. 611-5.
4. Lee, B.H., et al., *Enhancement of proteasome activity by a small-molecule inhibitor of USP14.* Nature, 2010. **467**(7312): p. 179-84.
5. Lam, Y.A., et al., *Editing of ubiquitin conjugates by an isopeptidase in the 26S proteasome.* Nature, 1997. **385**(6618): p. 737-40.
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7. Jacobson, A.D., et al., *The lysine 48 and lysine 63 ubiquitin conjugates are processed differently by the 26s proteasome.* J Biol Chem, 2009. **284**(51): p. 35485-94.