K63-linked Tetraubiquitin-Rhodamine 110

Cat. # SI233

LifeSensors www.lifesensors.com

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 which are also associated with the 19S regulatory particle, Rpn11-mediated 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 as a source of enzyme and ubiquitinated protein as a substrate, methods requiring separation of cleaved from uncleaved substrate for the 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].

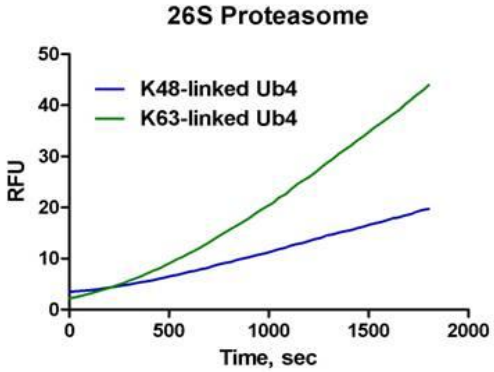
Application(s)

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

Product Specifications

Tag	None
Purity	≥ 90% by RP-HPLC
Molecular Weight	34,602.7 Da (calculated)
Quantity	50 µg
Species	Human
Expression System	*E. Coli*
Physical State	Lyophilized powder
Solubility	Soluble at 50 µM in PBS or TBS
Ex/Em wavelengths	Excitation: 485nm; Emission: 535nm
Storage	-80° C. Avoid repeated freeze/thaw cycles

Product QC

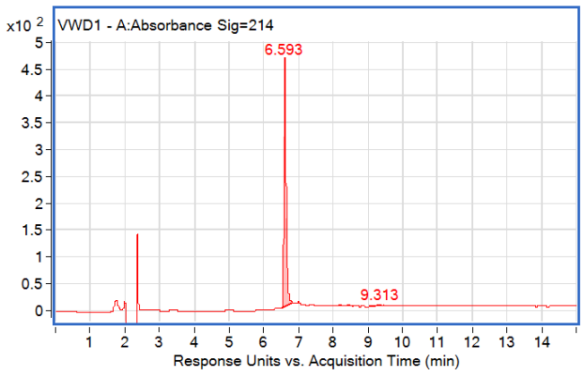


Isopeptidase activity of the 26S proteasome was measured with K48-linked tetraubiquitin-rhodamine 110 (Cat. # SI238; K48-linked Ub4; blue) and K63-linked tetraubiquitin-rhodamine 110 (Cat. # SI233; K63-linked Ub4; green) at 100nM as the increase in fluorescence at 535nm over time.

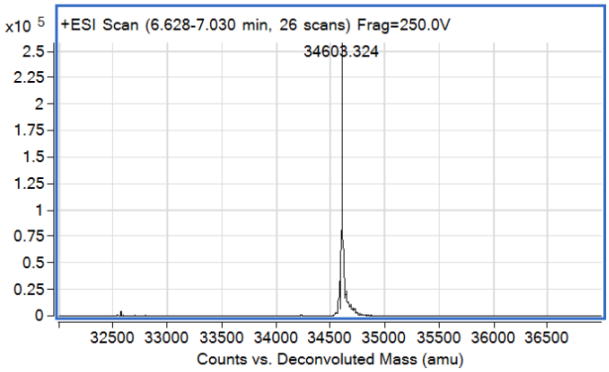
All products are for research use only • Not intended for human or animal diagnostic or therapeutic uses
Copyright © 2025 LifeSensors, Inc. All Rights Reserved

K63-linked Tetraubiquitin-Rhodamine 110

Cat. # SI233



RP-HPLC



Deconvoluted mass spectrum

References

1) Lee, M.J., et al., Mol Cell Proteomics, 2011; 10(5): p. R110 003871.
2) Yao, T. and R.E. Cohen., Anal. Biochem. Nature, 2002. 419(6905): p. 403-7.
3) Verma, R., et al., Science, 2002. 298(5593): p. 611-5.
4) Lee, B.H., et al., Nature, 2010. 467(7312): p. 179-84.
5) Lam, Y.A., et al., Nature, 1997. 385(6618): p. 737-40.
6) Koulich, E., X. Li, and G.N. Mol Biol Cell, 2008. 19(3): p. 1072-82.
7) Jacobson, A.D., et al., J Biol Chem, 2009. 284(51): p. 35485-94.

All products are for research use only • Not intended for human or animal diagnostic or therapeutic uses
Copyright © 2025 LifeSensors, Inc. All Rights Reserved