

DISCOVER MORE:  
IQF-substrates for measuring  
true isopeptide bond cleavage.

**The geometry of an isopeptide bond is dramatically different from that of a linear peptide bond, so why use a linear molecule, like ubiquitin-rhodamine, to measure isopeptidase activity?**

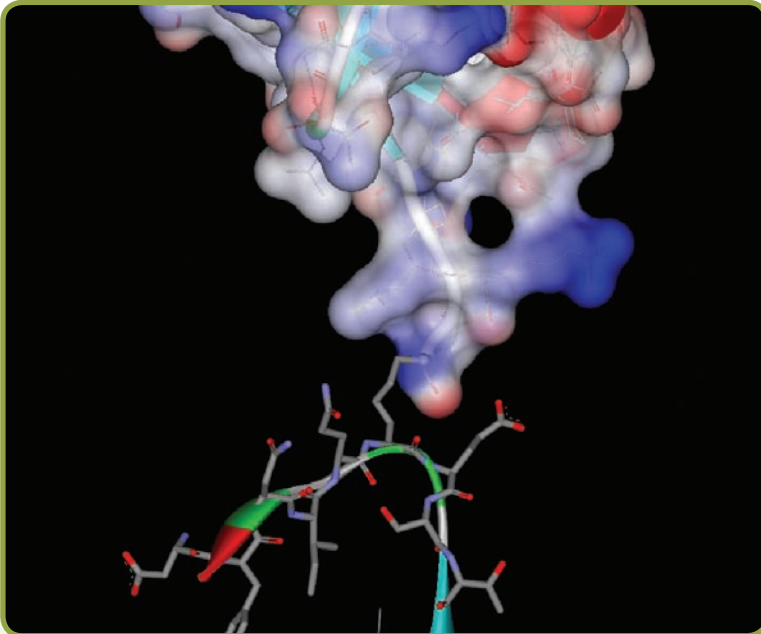
An axiom of high-throughput screening is that the quality of your hits are only as good as the ability of your assay to mimic nature.

As corollary to this axiom, your assay's ability to mimic nature is only as good as your substrate.

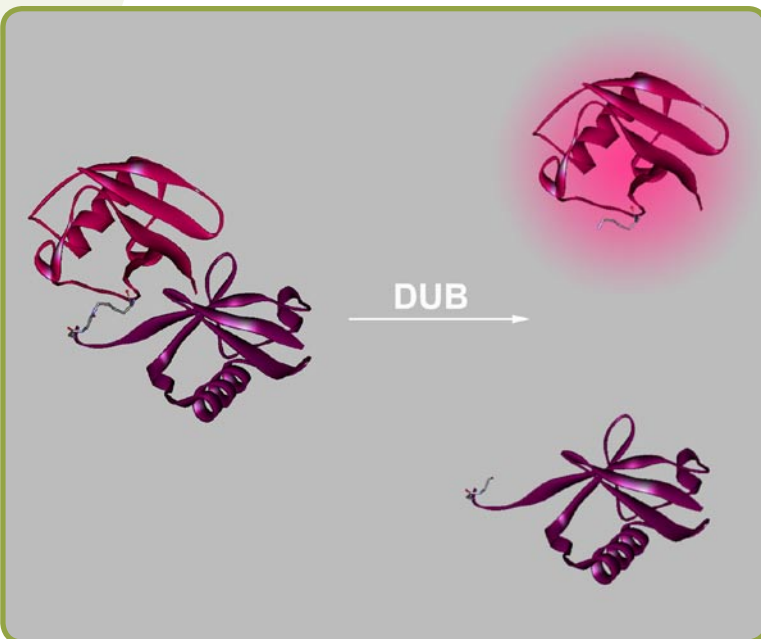
Choose the best substrate  
for your DUB.

LifeSensors' internally quenched  
fluorescence-diubiquitins (IQF-DiUb™):

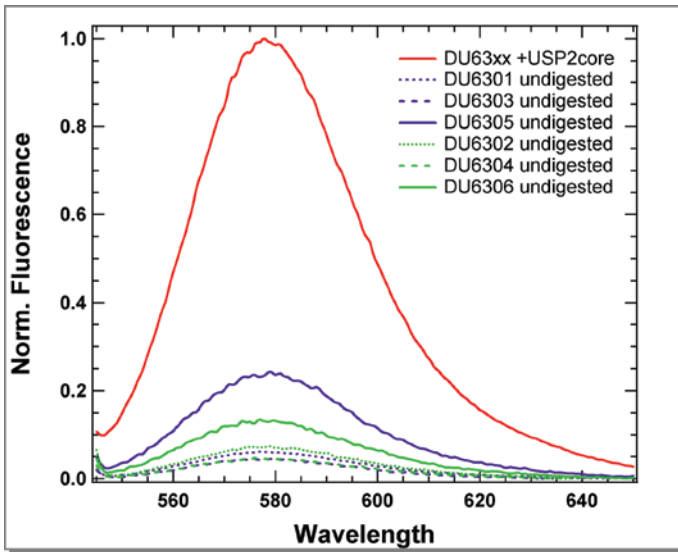
- Provide convenient, continuous readouts of isopeptidase activity.
- A bright, red-shifted fluorophore (TAMRA) to avoid interference from UV absorbing compounds
- A strong quencher to suppress TAMRA fluorescence in the intact diubiquitin
- Excellent signal to background ratios
- Native N-termini to preserve ubiquitin structure



K63-linked diubiquitin



Signal: Background depends on the location and relative distance of quencher and fluorophore.



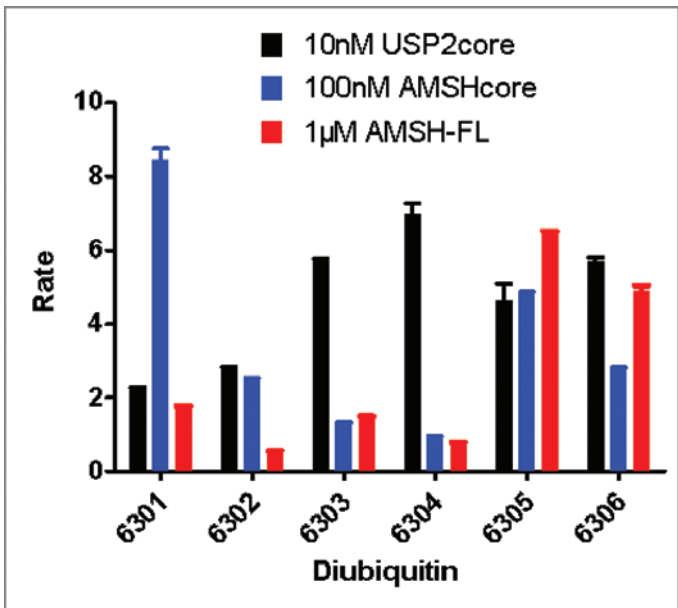
Fluorescent yield before and after digestion with USP2core (Exc. 540 nm)

Three quencher positions and two TAMRA positions yield six different diubiquitins\*.

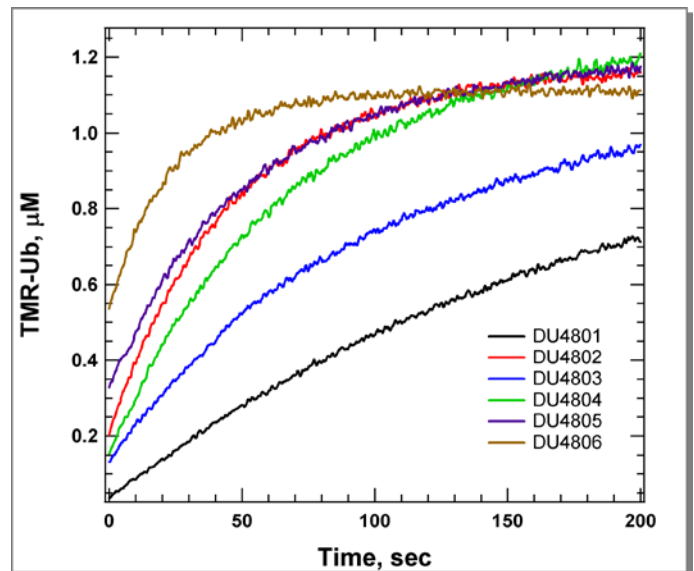
Signal:Background Ratios		
Variant	Linkage	
	K48	K63
01	12.7	16.7
02	13.6	14.1
03	20.7	22.7
04	12.7	22.2
05	6.0	4.3
06	4.6	7.7

\*Over 24 variants available

### Quencher/fluorophore positions affect DUB recognition and cleavage rates



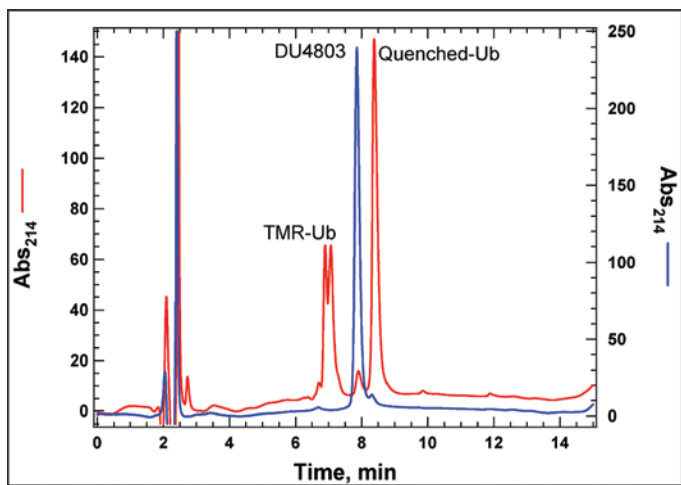
Rates of cleavage of K63-linked DiUbs by USP2core and AMSH.



Cleavage of K48-linked DiUbs by USP2core

Linkage specific DUB cleavage.  
 AMSh: K63; USP2: nonspecific; Otub1: K48; BAP1: C-term.

	AMShc	USP2c	Otub1	BAP1
DU4801	-	+++	-	-
DU4803	-	+++	+	-
DU4804	-	+++	+	-
DU6301	++	+++	-	-
DU6302	++	+++	-	-
DU6303	+	+++	-	-

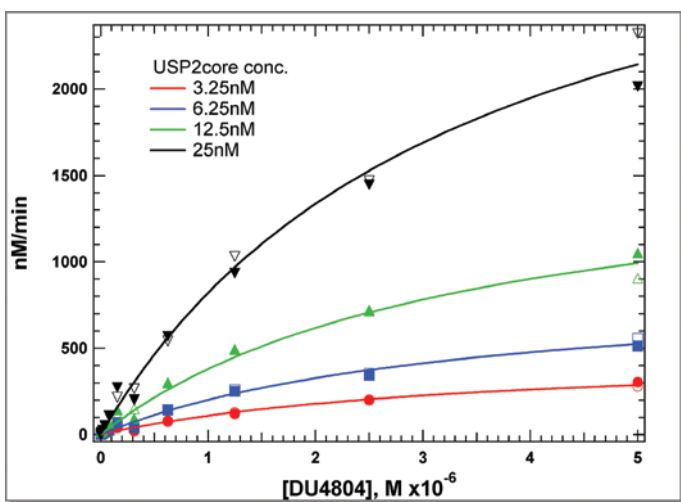


IQF-DiUb characteristics:

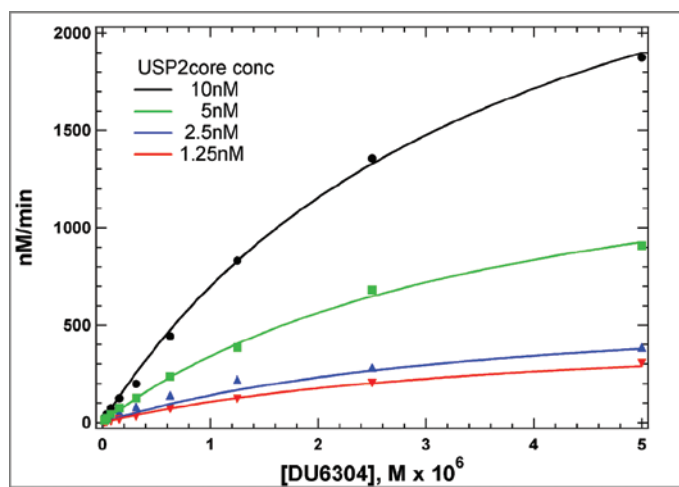
- High purity
- Virtually 100% cleavable
- Zero background drift over time
- Z' values >0.7 in 384-well assays

Cleavage of DU4803 by USP2core

Rapid determination of kinetic constants with multiple enzymes over wider enzyme and substrate ranges when compared to gel or LC/MS analyses



$$k_{cat}/K_m = 0.7 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$$



$$k_{cat}/K_m = 1.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$$

**Contact Us  
To Learn More:  
info@lifesensors.com**

To order, please call  
610-644-8845 or visit  
www.lifesensors.com

## LifeSensors IQF-DiUb™ are:

- Currently available in K48- or K63-linked forms
- Additional linkages – K11, K6, K29 – coming soon
- Priced competitively with Ub-rhodamine and Ub-AMC
- Available as a package with our DUB profiling service
- Bulk pricing available

The geometry of an isopeptide bond is dramatically different from that of a linear peptide bond, so why use a linear molecule, like ubiquitin-rhodamine, to measure isopeptidase activity?

### Recommended Workflow:

- Determine optimum substrate using our K48 or K63 panels
- Order the best substrate for your DUB
- Get RESULTS!

Part #	Product Name	Product Description	Linkage	Variant	QTY	Price
<b>48 Variants</b>						
DU4801	DiUb48-1	IQF DiUbiquitin Substrate	48	1	50ug	\$275 USD
DU4802	DiUb48-2	IQF DiUbiquitin Substrate	48	2	50ug	\$275 USD
DU4803	DiUb48-3	IQF DiUbiquitin Substrate	48	3	50ug	\$275 USD
DU4804	DiUb48-4	IQF DiUbiquitin Substrate	48	4	50ug	\$275 USD
DU4805	DiUb48-5	IQF DiUbiquitin Substrate	48	5	50ug	\$275 USD
DU4806	DiUb48-6	IQF DiUbiquitin Substrate	48	6	50ug	\$275 USD
<b>63 Variants</b>						
DU6301	DiUb63-1	IQF DiUbiquitin Substrate	63	1	50ug	\$275 USD
DU6302	DiUb63-2	IQF DiUbiquitin Substrate	63	2	50ug	\$275 USD
DU6303	DiUb63-3	IQF DiUbiquitin Substrate	63	3	50ug	\$275 USD
DU6304	DiUb63-4	IQF DiUbiquitin Substrate	63	4	50ug	\$275 USD
DU6305	DiUb63-5	IQF DiUbiquitin Substrate	63	5	50ug	\$275 USD
DU6306	DiUb63-6	IQF DiUbiquitin Substrate	63	6	50ug	\$275 USD
<b>Test Panels (Use to determine optimal optimal variant of either 48 or 63 specific linkage)</b>						
DU0101	DiUb48 Panel	IQF DiUbiquitin Panel	48	1-6	25 uL of 20 uM each	\$195 USD
DU0102	DiUb63 Panel	IQF DiUbiquitin Panel	63	1-6	25 uL of 20 uM each	\$195 USD
DU0201	DiUb48/63 Panel	IQF DiUbiquitin Panel	48/63	1-6(48), 1-6(63)	25 uL of 20 uM each	\$345 USD

Custom DiUbiquitin Substrates are available. Please contact LifeSensors for more information.

### About LifeSensors, Inc.

LifeSensors is a biotechnology company located 35 miles west of Philadelphia, Pennsylvania, USA. Founded in 1996, LifeSensors has developed a number of innovative protein expression technologies that enable efficient translation of the genome into proteome. LifeSensors is well-known for its innovations in an important family of proteins consisting of ubiquitin and ubiquitin-like proteins (UBL) such as SUMO (Small Ubiquitin-like MOdifier). LifeSensors has been granted several patents to cover the use of SUMO and other UBLs as gene fusion tags to improve the expression and purification of recombinant proteins. Additional patent applications are in various stages of review. Currently, LifeSensors is expanding its protein production capabilities and is developing protein microarrays for drug discovery and diagnostics.

IQF-DiUb™ substrates (patent pending) were co-developed with Progenra, Inc.

all products are for research use only not intended for human or animal diagnostic or therapeutic uses

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