DUB Screening & Profiling

LifeSensors

271 Great Valley Parkway

Malvern PA 19355

Phone: 610-644-8845

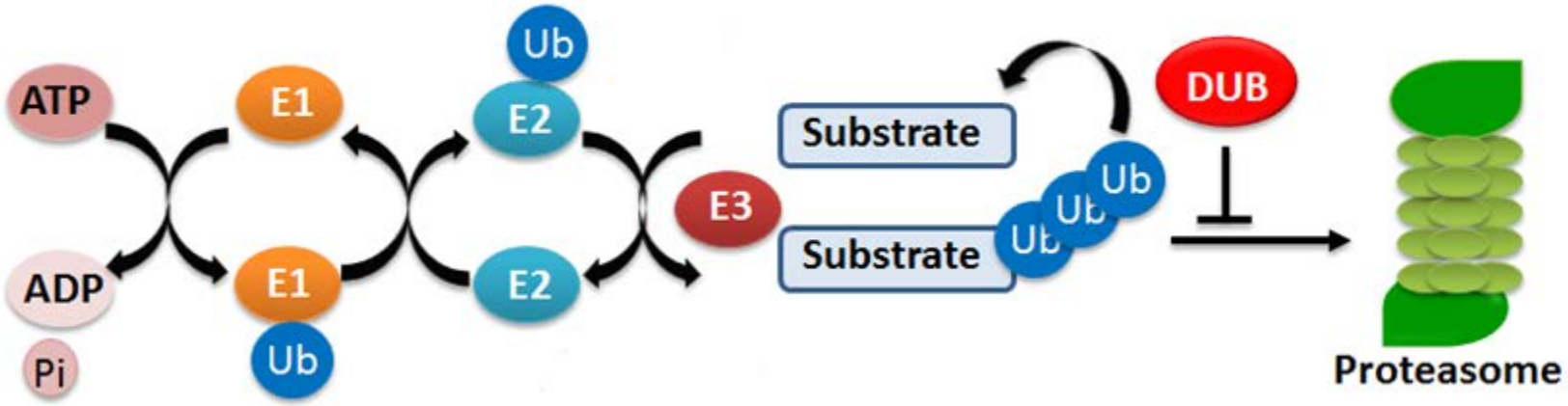
bd@lifesensors.com

www.lifesensors.com

LifeSensors

- Leading Biotech in UPS Drug Discovery and Diagnostic R&D
- ~500 Products, Proteins, Ubiquitin Affinity Reagents (TUBEs), Inhibitors, Assays, Kits and Proprietary Protein Expression Systems (SUMO)
- Drug Discovery, UPS and PROTAC Screening Services
- Profiling Compounds Against Ubiquitin Ligases and DUBs
- Custom Assay Development and Collaborative Research

Ubiquitin Proteasome System



E1 – Ubiquitin activating enzyme

Requires ATP to attach Ub to E1

E2 – Ubiquitin conjugating enzyme

Transfers Ub from E1 to E3

E3 – Ubiquitin ligases

Transfers Ub to self or substrate

Forms mono-Ub or poly-Ub chains

DUB – Deubiquitinase

Removes mono-Ub or poly-Ub chains

Proteasome – Degrades ubiquitylated proteins

DUB-based Drug Discovery Capabilities

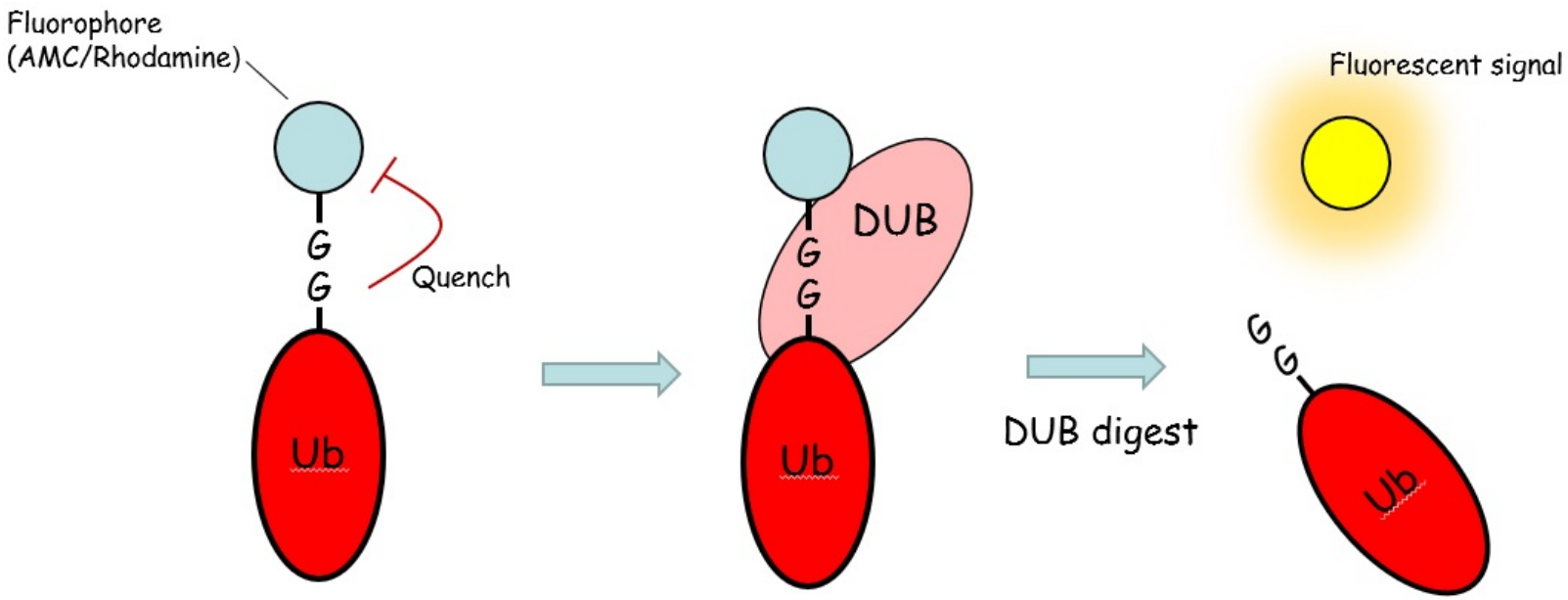
- ~40 biologically active DUBs
- Developed ~25 DUB assays for a variety of HTS and validation strategies
- Ability to screen ~500,000 compounds
- Enzyme selectivity panels and compound profiling
- Determine compound MOA, cellular and target tissue PD markers
- Enabling technologies based on TUBE applications

LifeSensors DUBs

DUB Family	Representative DUBs
USP	USP2, USP5, USP7, USP8, USP15, USP20, USP21, USP30, USP34, USP47, USP51
OTU	OTUB1, Cezanne,
UCH	UCHL1, UCHL3, UCHL5
Viral & Bacterial	PLPro, PLP2, Ssel
MJD	Ataxin 3, JosD1
JAMM	AMSH
DeSUMOylase	Ulp1, SENP2, SENP6
DeNEDDylase	Den1
MINDY	MINDY1

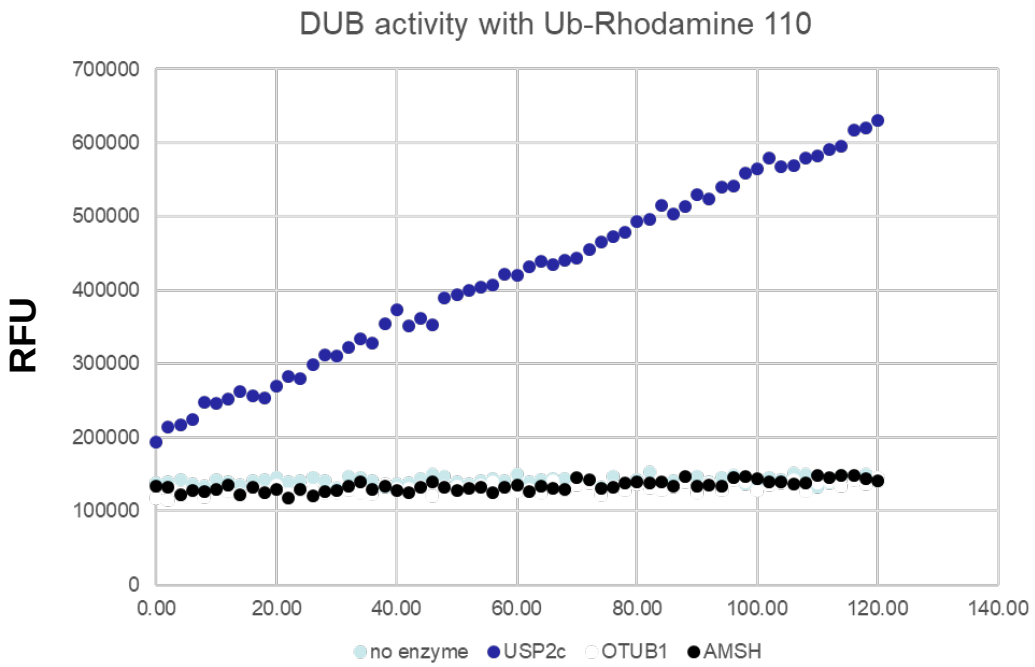
Step One: DUB Assay Development, Optimization and HTS

DUB HTS and Validation Assays

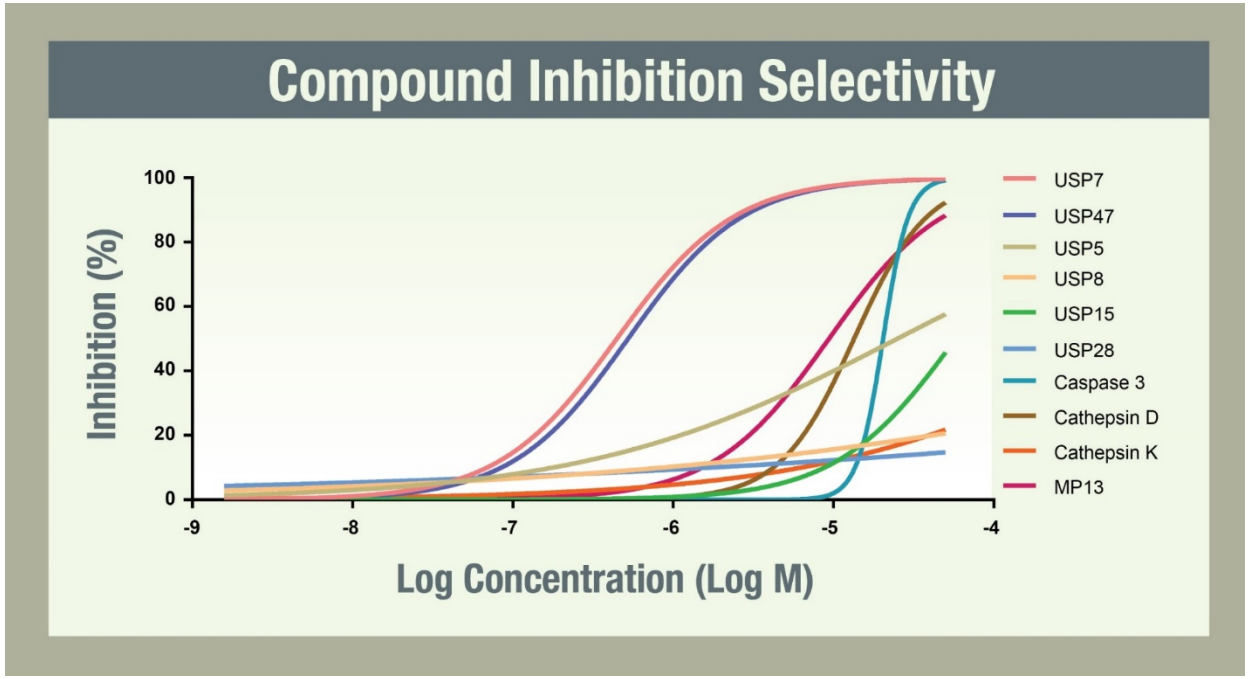


The **Ubiquitin Fluorophore Assay** utilizes a ubiquitin molecule bound to a quenched fluorophore (e.g. Rhodamine 110). After DUB digestion the unconjugated fluorophore is detected by measuring the change in fluorescence on a plate reader. This simple and straight-forward assay is a great high-throughput option for most DUBs.

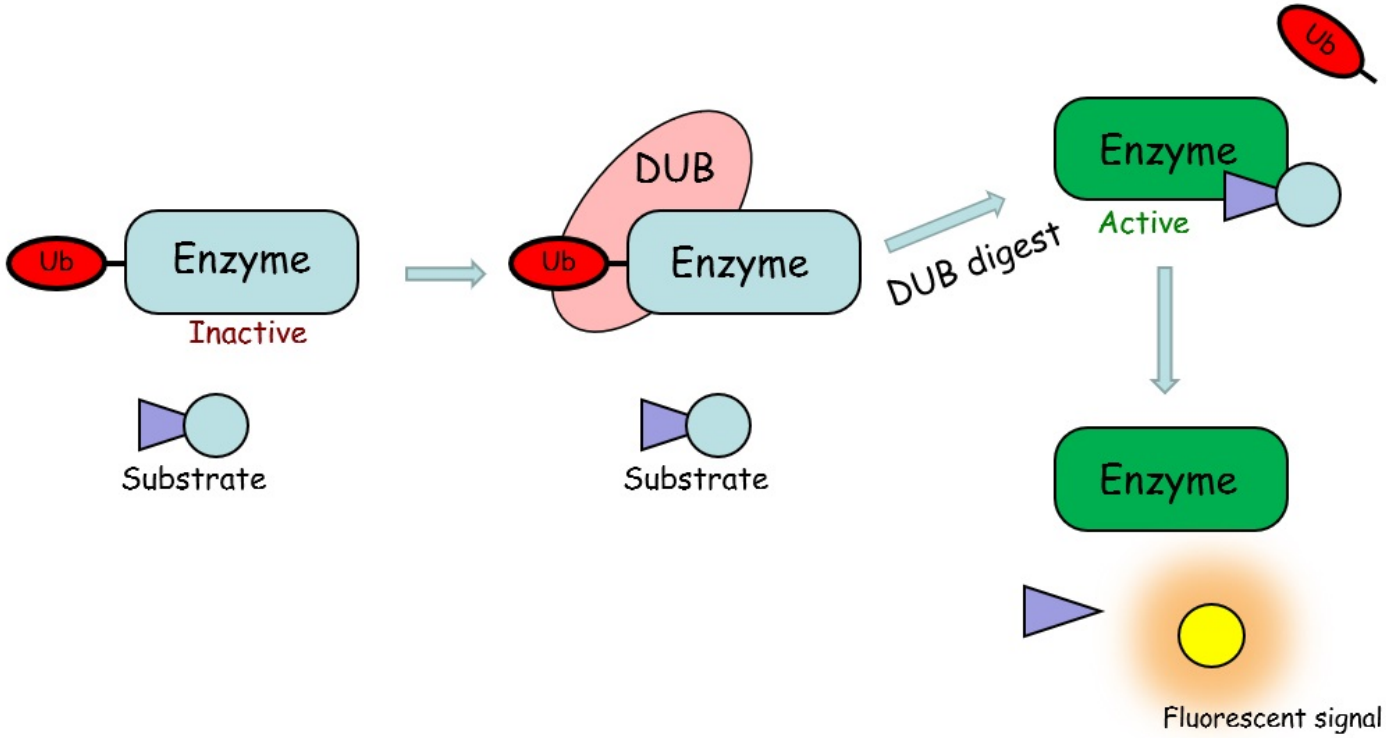
Example of DUB HTS and Validation Assays



DUB activity with Ub-Rho 110 in the presence of a compound specific for USP7



DUB HTS and Validation Assays

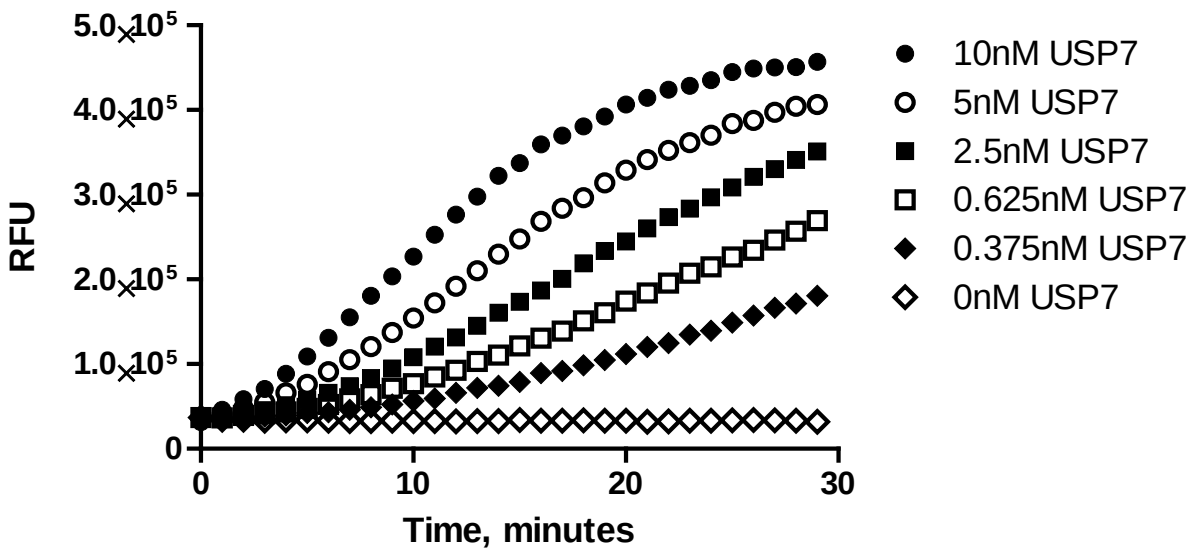


The **Ub-CHOP-Reporter Deubiquitylation Assay Kit** consists of ubiquitin fused to a reporter enzyme. Fusion of the N-terminus of the reporter to the C-terminus of ubiquitin renders the reporter catalytically inactive. Following cleavage of the Ub-reporter system by DUBs, the free (and now active) reporter enzyme acts on its fluorescence substrate. Thus, in this coupled assay, the signal generated by cleavage of the reporter enzyme's substrate is a quantitative measure of DUB activity.

Example of DUB HTS and Validation Assays

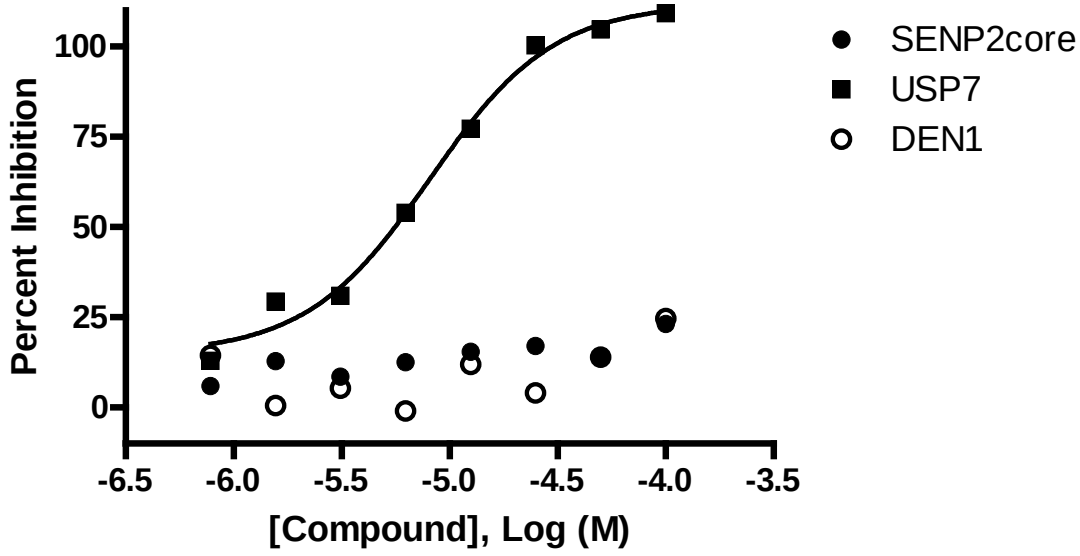
CHOP Assay

Dose response of USP7 in CHOP assay



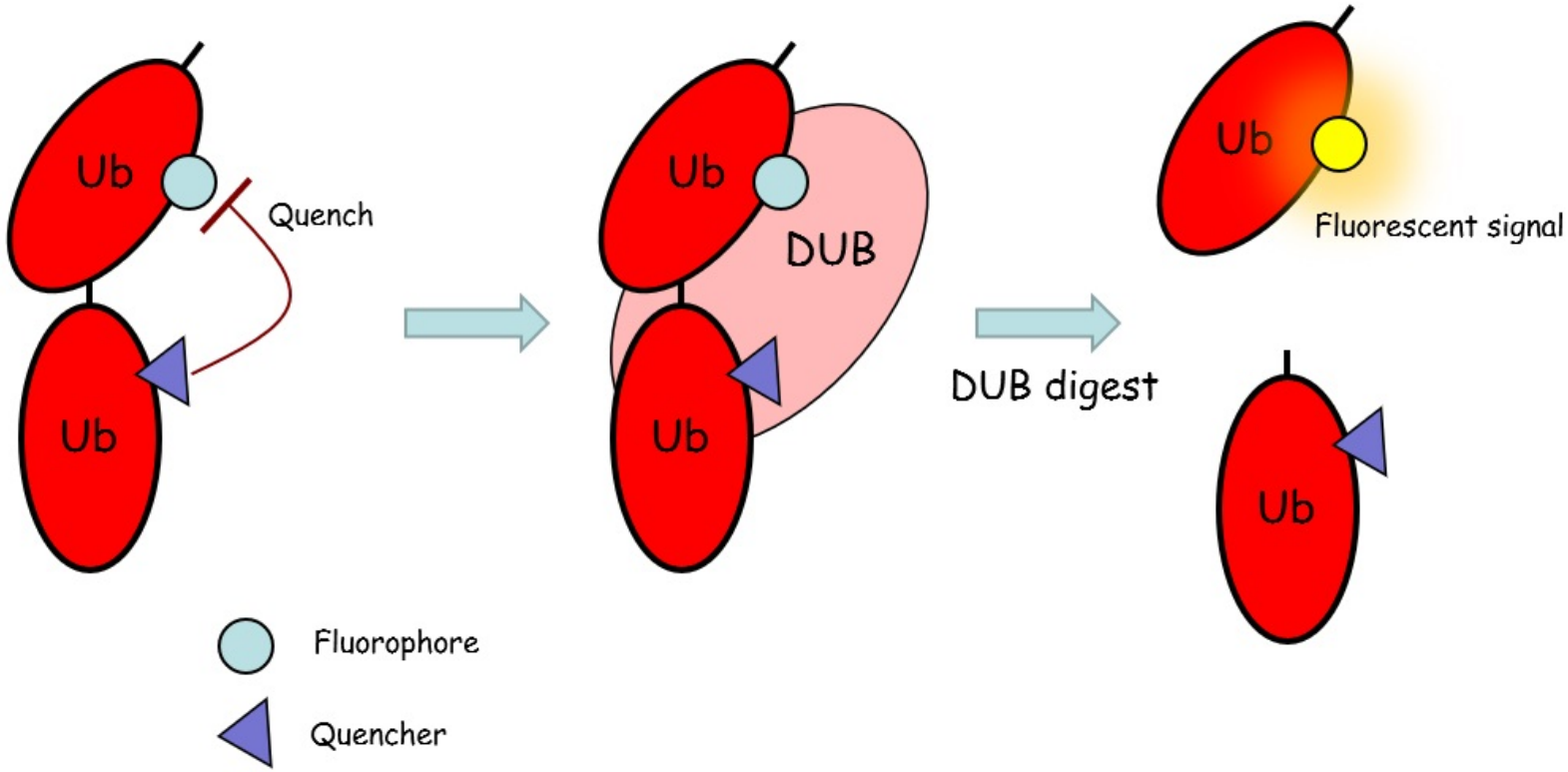
IC50 values of USP7 inhibitor compound X

SENP2core and DEN1 are negative controls



LifeSensors from genomics to proteomics

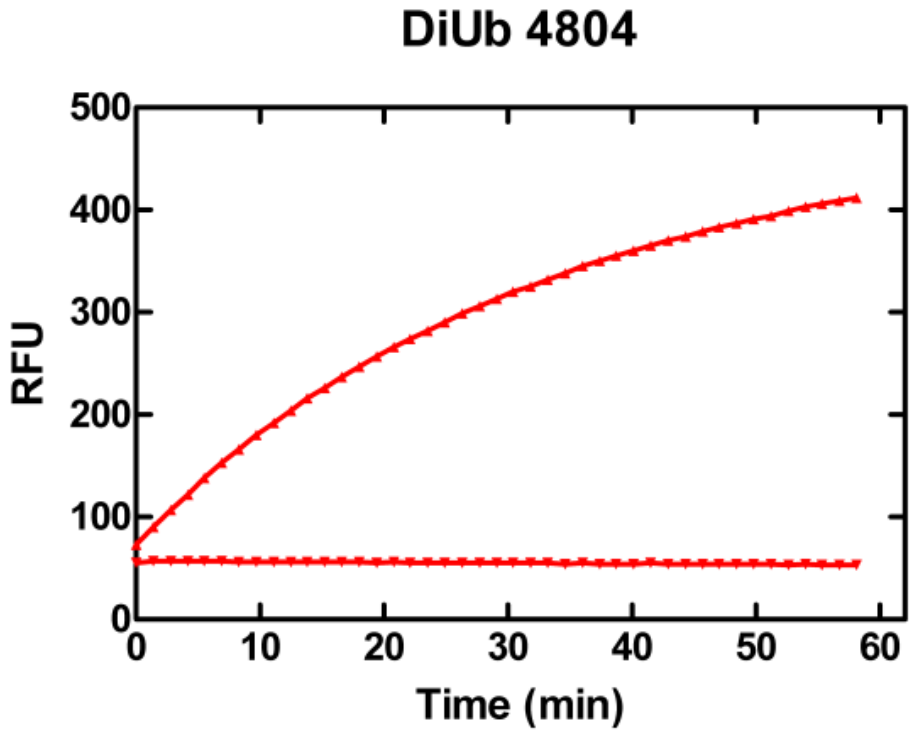
DUB HTS and Validation Assays



The **IQF Assay** utilizes a pair of conjugated ubiquitin proteins. One carries a fluorophore silenced by the nearby quencher. After DUB digestion the free fluorophore can be detected by a plate reader. This assay is physiologically relevant since the DUB cleaves a Ub-Ub bond.

Example of DUB HTS and Validation Assays

Internally quenched fluorescent FRET pair (IQF) assay



Progression of DiUb cleavage by USP2core

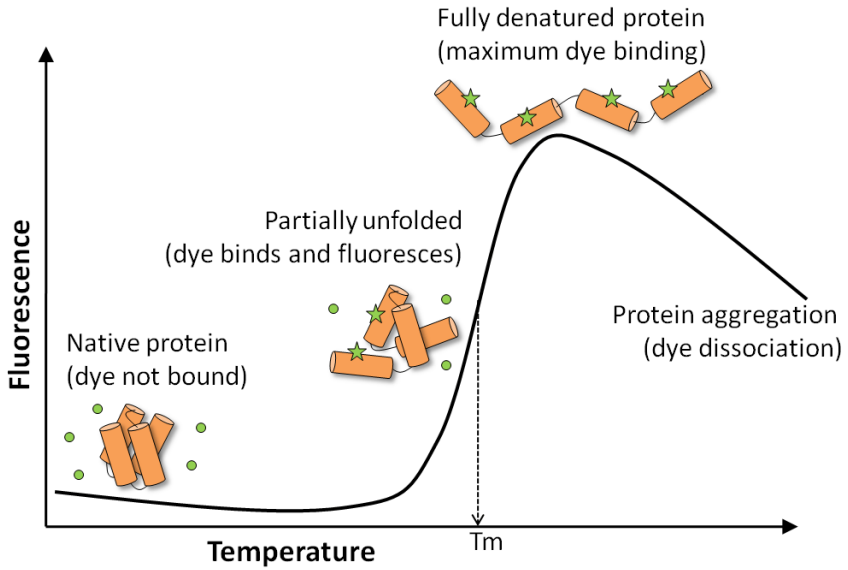
DiUb K48-4 (200 nM) was incubated with (closed symbols) or without (open symbols) 10nM USP2core. The Increase in TAMRA fluorescence was monitored.

Step Two: Hit-to-lead optimization

Validation Assays

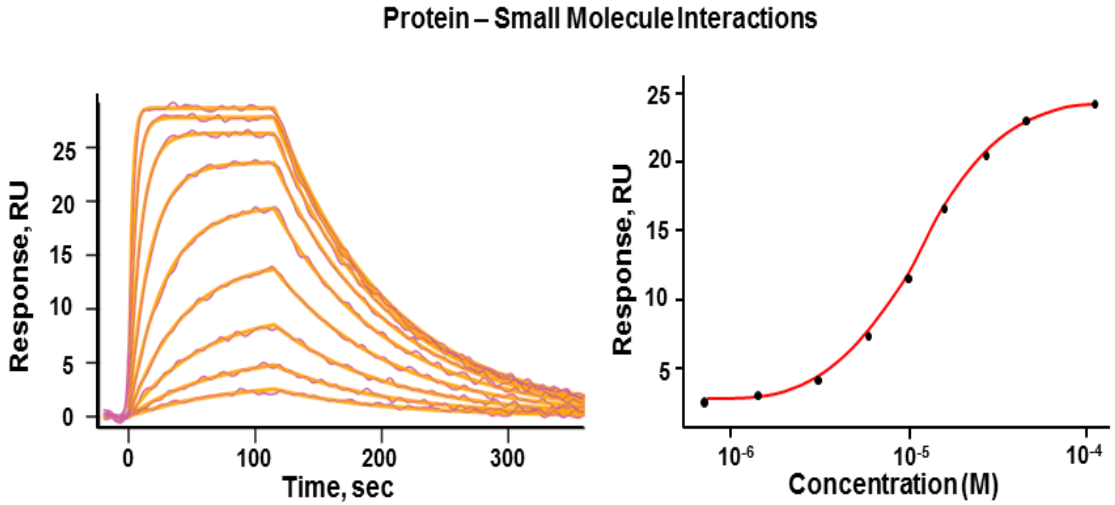
Thermal Shift Assay

HTS assay to detect compound binding to a target



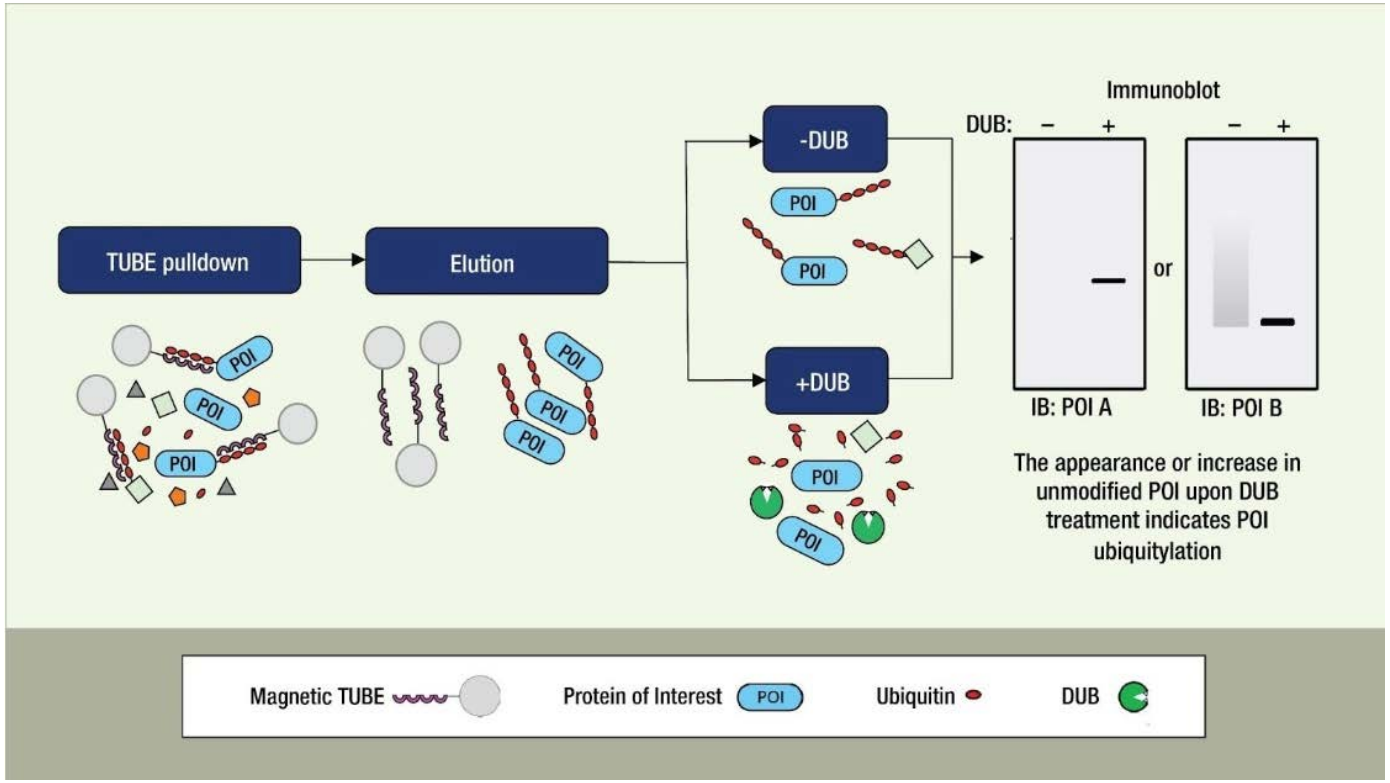
Surface Plasma Resonance

Determination of a small molecule affinity to a target



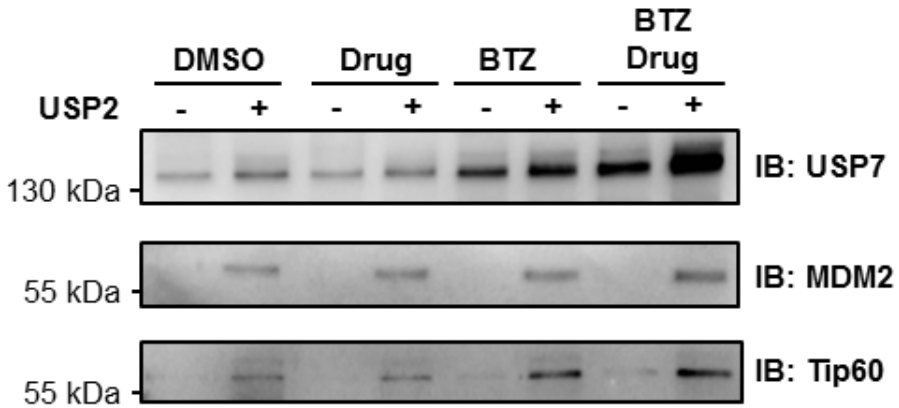
LifeSensors from genomics to proteomics

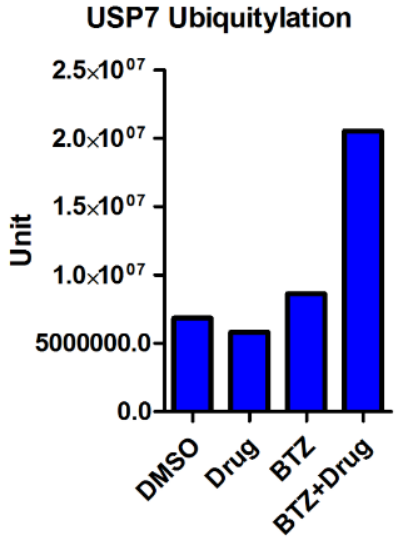
UbiTest: Cellular Assay to Measure Ubiquitylation of POI

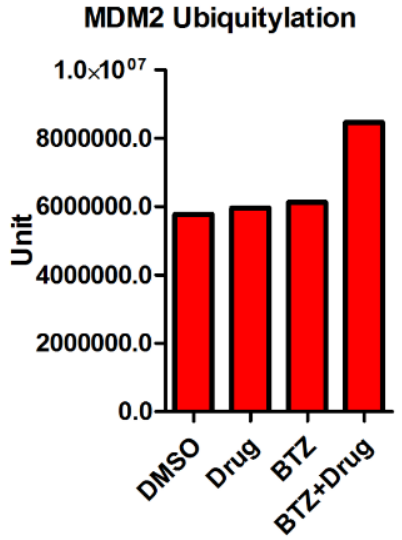


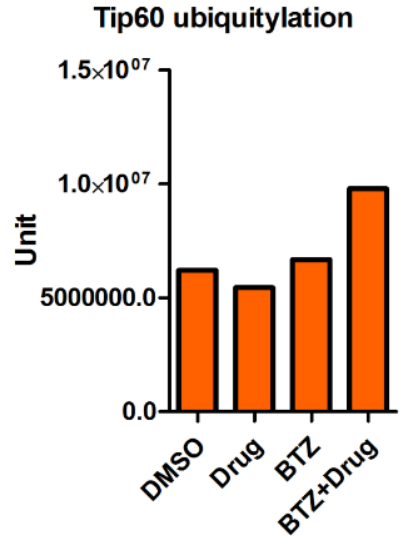
UbiTest – a TUBE-based pull-down method that isolates total cellular ubiquitylated proteins. Subsequently, samples are treated with pan-selective DUBs to remove polyubiquitin chains. The target protein is identified by its native molecular weight and analyzed and quantified by immunoblotting. UbiTest is one of the most sensitive methods available to quantify ubiquitylation levels of proteins in vivo.

Example of UbiTest Assay









Determine endogenous target protein ubiquitylation using UbiTest

Jurkat cells were treated with indicated compounds and lysed in RIPA buffer. Anti-Ub TUBE1 agarose resin was added for pull down total polyubiquitylated proteins and then elution was incubated with DUB. Immunoblot (left) of the assay is shown and quantitation (right) of the bands showed increased signal of USP7, MDM2, Tip60 after DUB treatment indicating they are polyubiquitylated.

LifeSensors DUB Selectivity Panel

(largest collection of functional DUBs in the industry and growing)

DUB Panel	Representative DUBs
Panel I (7 DUBs)	UCHL1, USP8c, USP15, USP7, USP4, USP2c, USP30.
Panel II (20 DUBs, includes DUB from panel I as well)	UCHL1, UCHL3, UCHL5, USP8c, USP15, USP7, USP4, USP2c, USP30,SENP1c, USP47, USP9x, USP21, USP10, OTUB1, YOD1, OTUB2, OTUD6B, Cezanne, A20.

Each DUB assay has been validated. LifeSensors profiles inhibitory or activation properties of every compound in Panel I followed by Panel II.

DUB Screening & Profiling Service

- Help customer discover DUB inhibitors or activators
- Express biologically active DUBs and substrates
- Develop and optimize HTS assay for DUB
- Screen in house libraries or customer libraries at LifeSensors
- Confirmation and counter screening to eliminate off-target compounds
- Biophysical and biochemical assay development for target engagement
- Cell-based assays to determine target engagement by compound
- All IP and data belong to the customer
- Work performed under CDA and Master Service Agreement
- Fee for service model, defined milestone-based agreement

Contact Us!

We are your partner for DUB drug discovery

Contact Information

Research & Product Inquiries	**R&D**	**info@lifesensors.com**	**610-644-8845 (ext 339)**
Custom Service & Assays	**BD**	**bd@lifesensors.com**	**610-644-8845 (ext 310)**

LifeSensors from genomics to proteomics