Elucidating the PROTAC Method of Action

Karteek Kadimisetty Ph.D

LifeSensors Inc.

271 Great Valley Parkway

Malvern PA 19355

Phone: 610-644-8845

bd@lifesensors.com

www.lifesensors.com

LifeSensors from genomics to proteomics

Summary

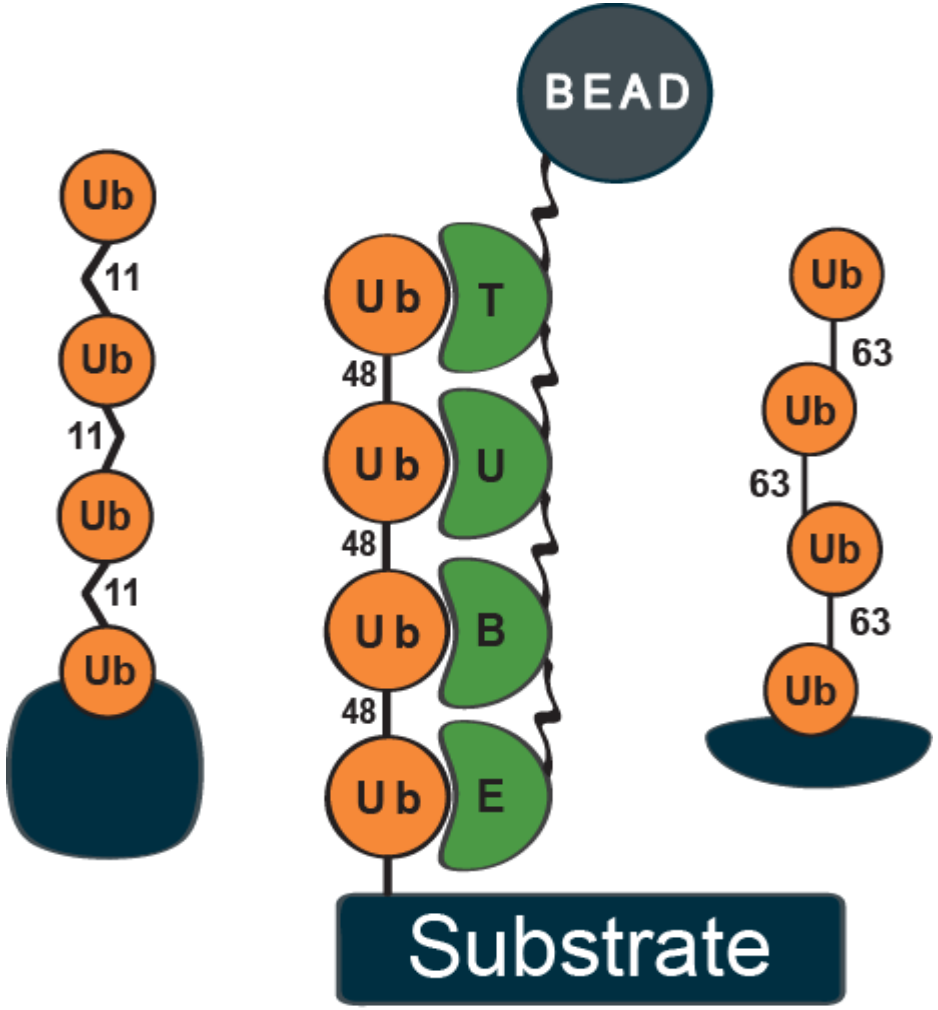
- **How to Speed Up PROTACs Discovery**
  - Blots are time consuming, non-quantitative, no dose response, time course or IC50!
  - Reporter genes cell lines don’t correlate with true function

- **Establishing a link between ubiquitylation and degradation**
  - Not all ligases function for degradation
  - Not all PROTACs degrade

- **Rapid in vitro PROTACs drug discovery**
  - Plate based, label free PROTACs discovery

Consult with LifeSensors for development of your custom PROTAC assay

TANDEM UBIQUITIN BINDING ENTITIES



- Isolation of ubiquitinated substrates from cell lysates
- Superior to antibodies, detection by Western blot
- E3 ligase and DUB assays using TR-FRET assay
- *In situ* detection with fluorescence
- Ubiquitin mass spec proteomics bypassing SILAC

Applications of TUBEs

Pan-Selective TUBEs (TUBE1 & TUBE2)
&
Linkage Specific TUBEs (K63,K48 & M1)

TUBE Applications

Pull-down of polyubiquitinated proteins

- Magnetic TUBEs
- GST TUBEs
- Agarose TUBEs
- His6 TUBEs

Bio-Markers

- In Vivo Drug Validation
- DUB Screening
- E3 Screening and Profiling

'Far Western' blot

- Biotin TUBEs

Mass Spectroscopy/ Proteomics

- TUBE Based M/S
- UbiSight Proteomics
- Microarrays for Ubiquitomics

Histochemistry/ Cytochemistry

- TAMRA-TUBEs
- Fluorescein TUBEs

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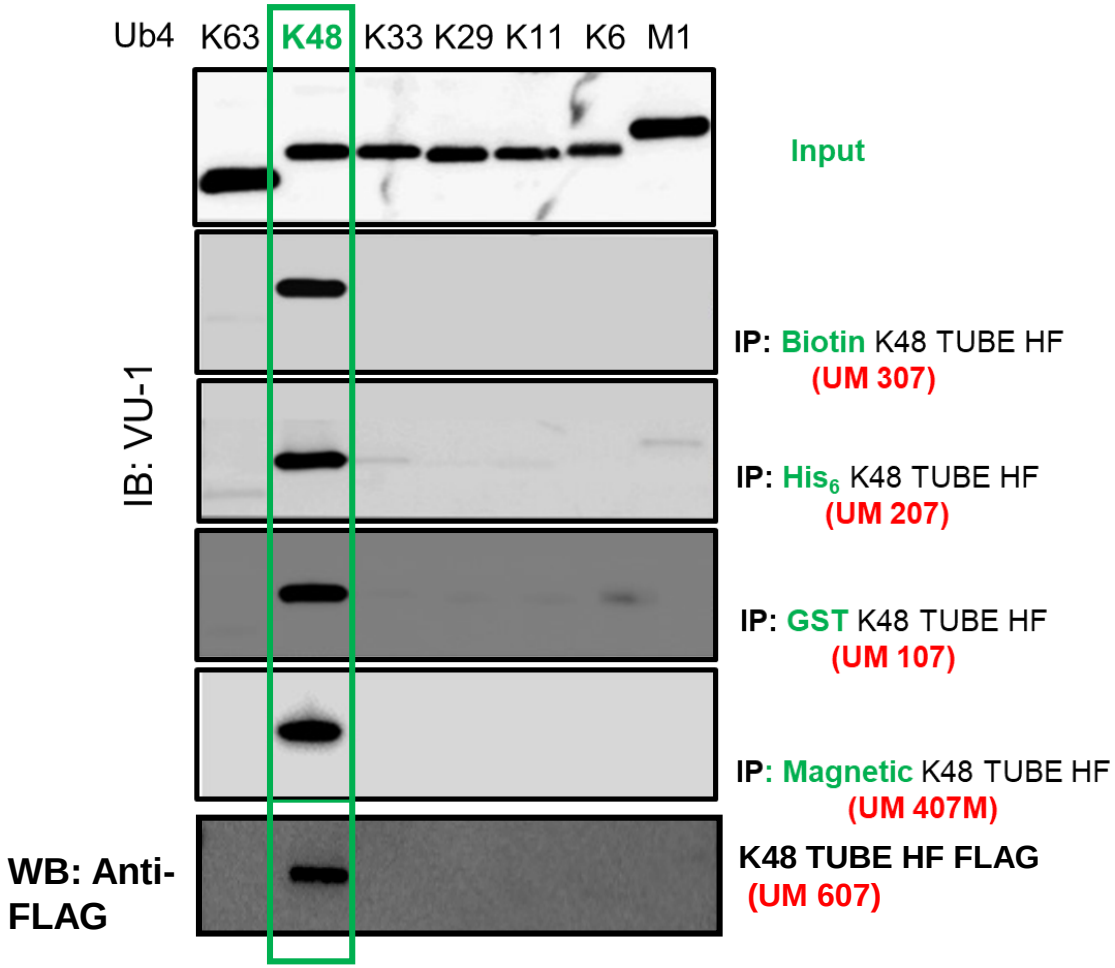
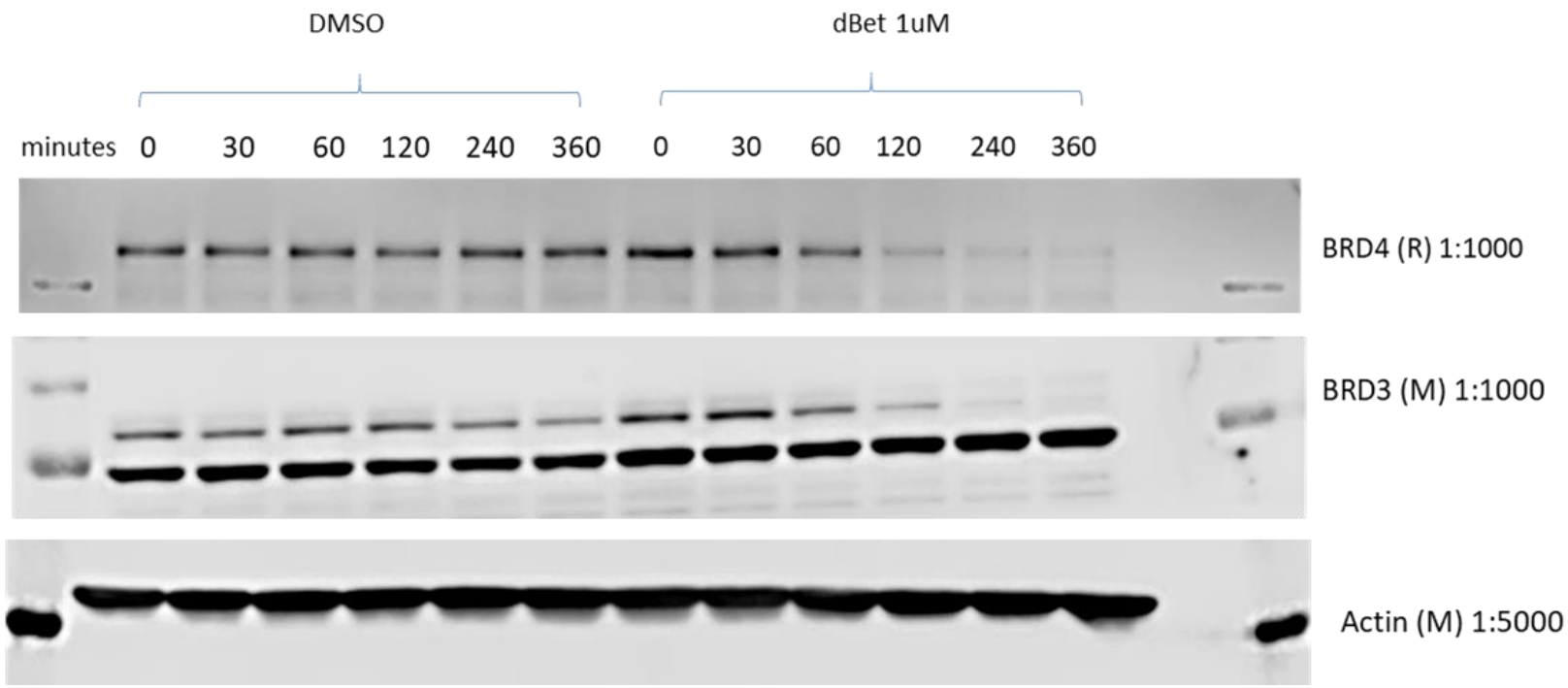
Enrichment/Detection of polyubiquitinated proteins



K48 TUBE HF FLAG detects only K48-linked ubiquitin chains in Western Blots

BRD3 and BRD4 Cereblon PROTAC Function, Time Course

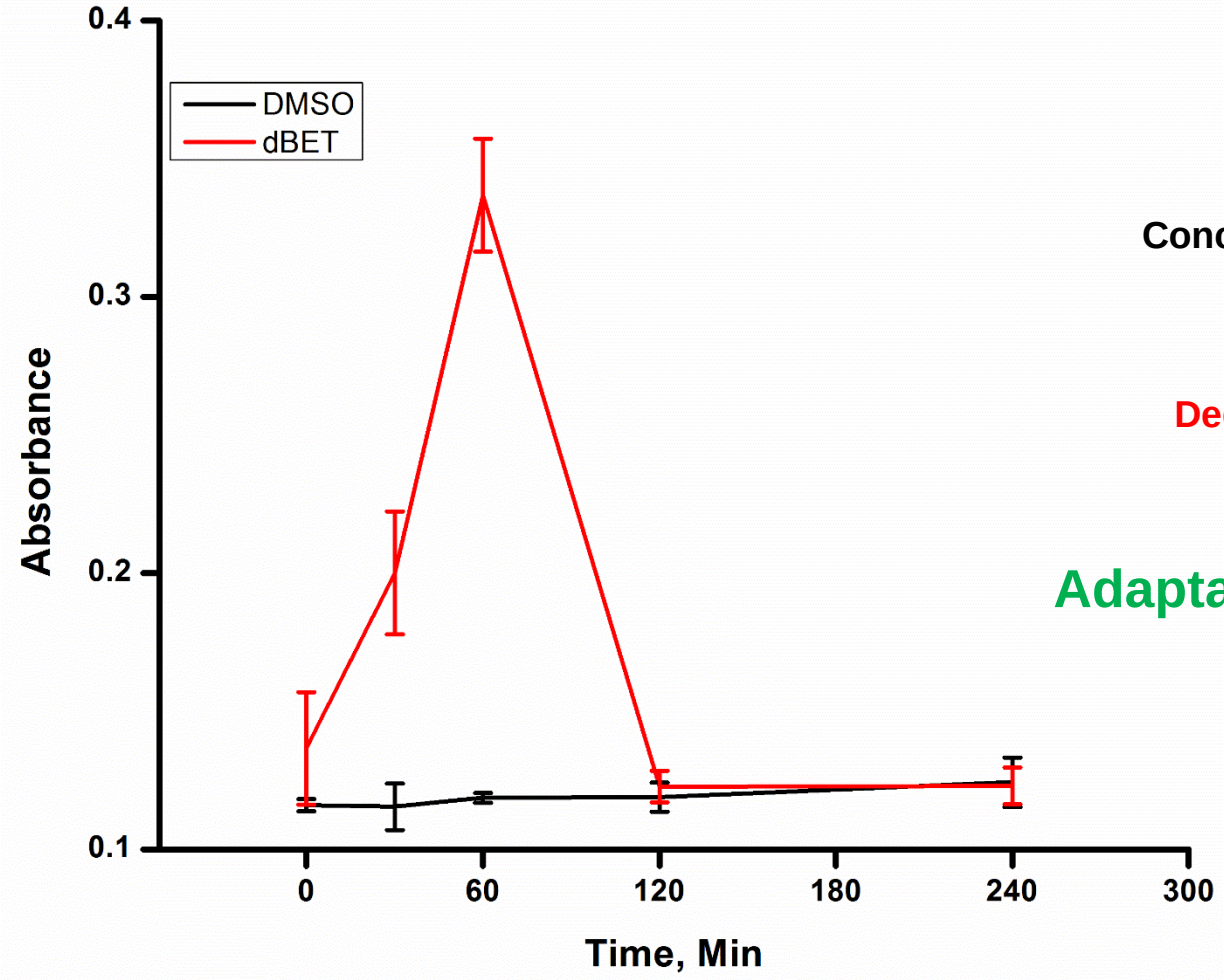
Jurkat Cells



Rapid Detection of BRD-3 Ubiquitination > dBET Treatment

Jurkat Cells

TUbiQuant™



Concentration dependent Assay:
Ubiquitination Rate

Correlate to
Degradation Rate, D_{max} , DC_{50}

Adaptable to other substrates

TUBE based Ubiquitinated protein pulldown on a plate and subsequent protein of interest detection

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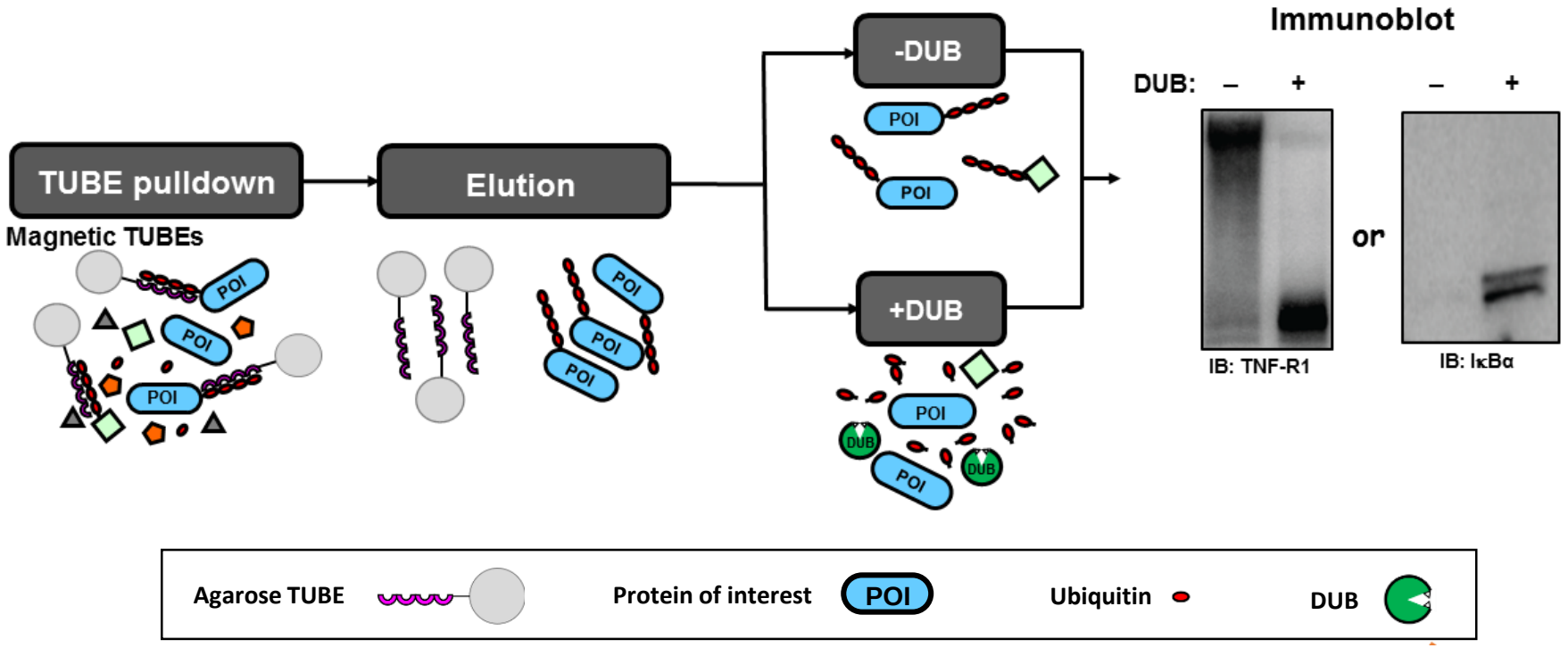
Assays for Cellular Ubiquitination Activity

- UbiTest

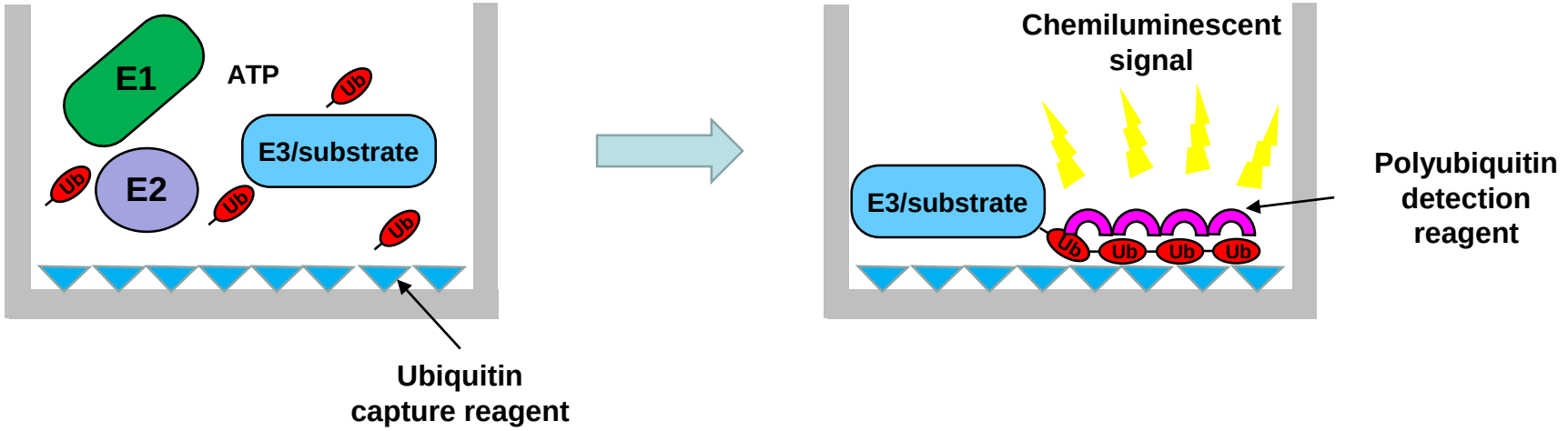
  Quantification of enzyme and substrate ubiquitination levels in a cellular context.



E3 Ligase HTS and Validation Assays

- E3 ELISA assay

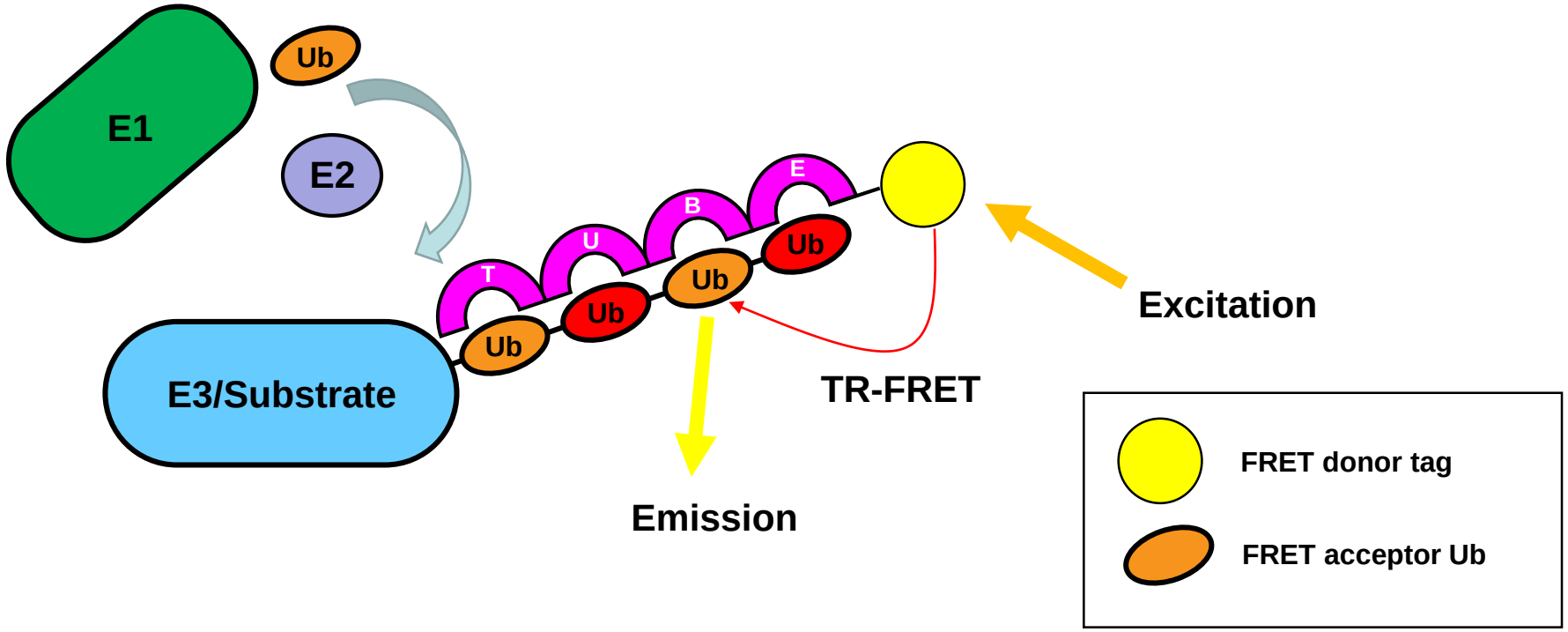
  Quantification of E3 ubiquitin ligase activity, employs a proprietary reagent to capture polyubiquitin chains formed in an E3 ligase dependent manner



E3 Ligase HTS and Validation Assays

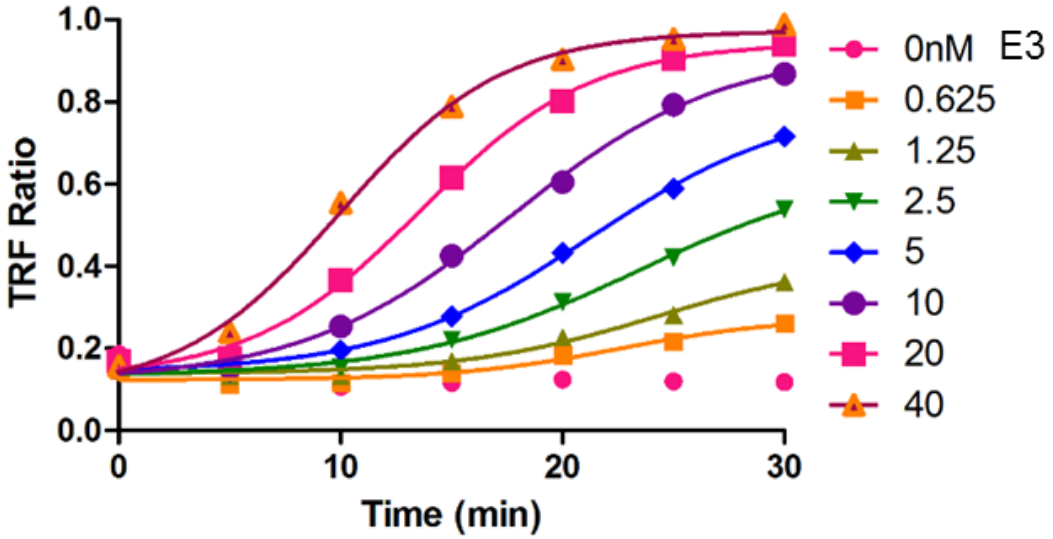
- TR-FRET E3 assay

  In vitro HTS assay to monitor substrate ubiquitination or E3 autoubiquitination in a TR-FRET format.

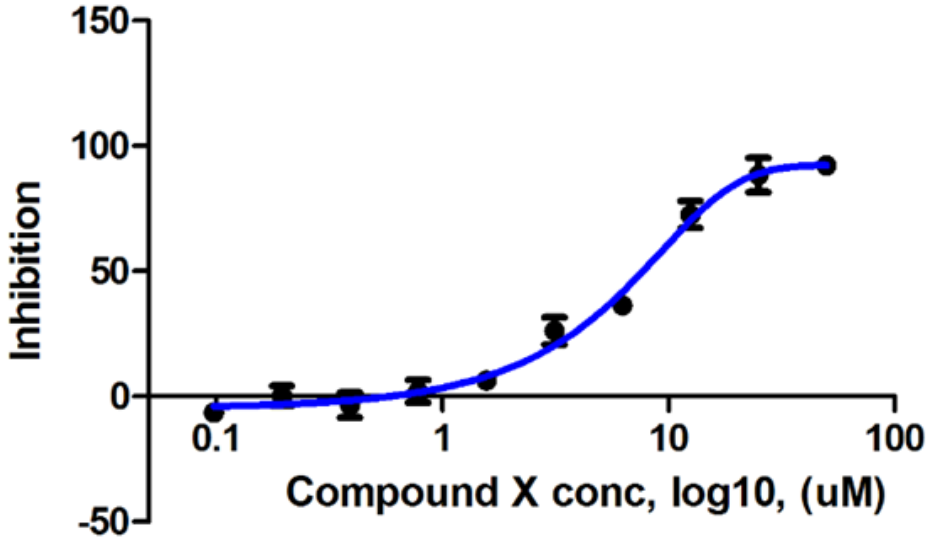


HTS TR-FRET E3 lite assay

E3 Titration
Protein X Ubiquitination

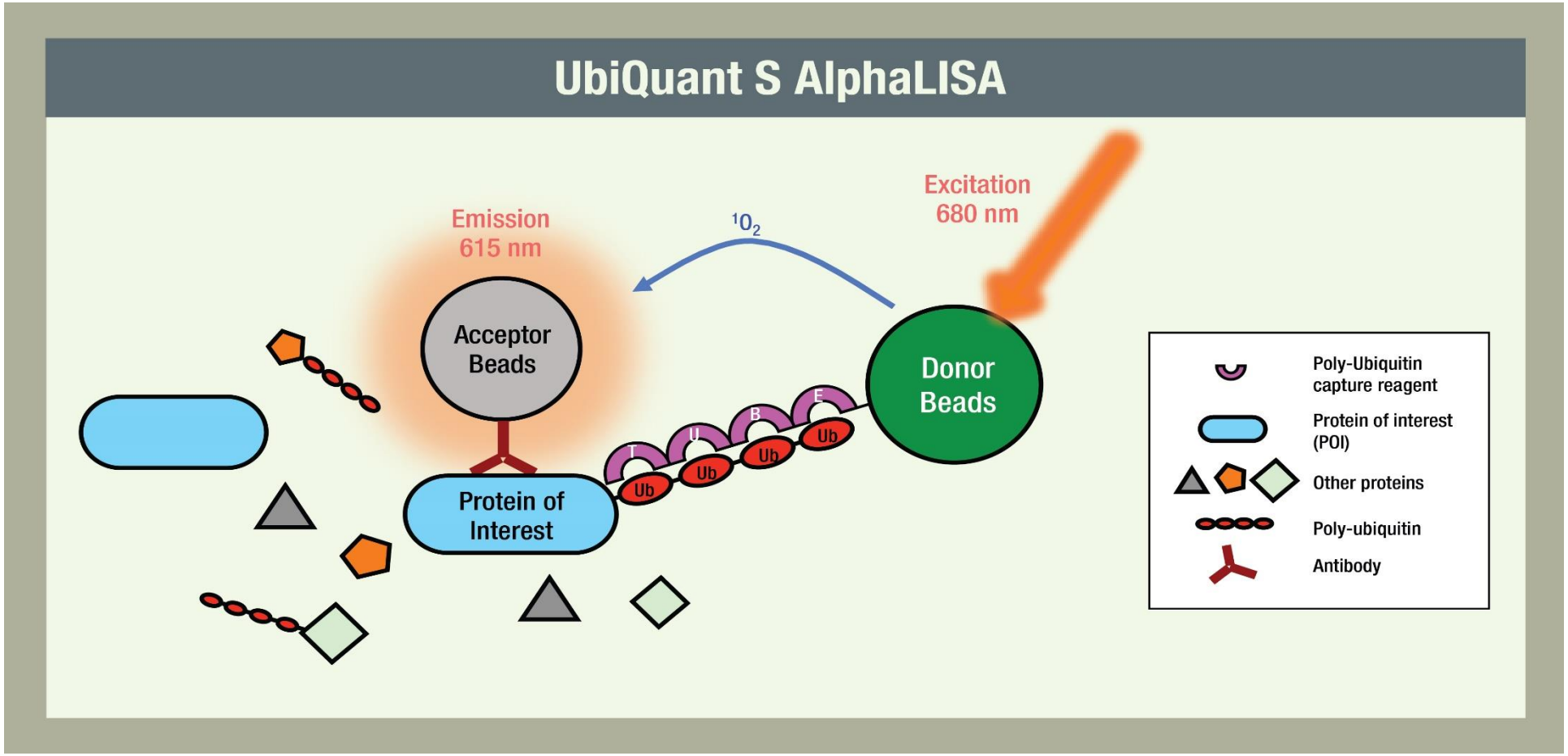


Protein X Ubiquitination
Inhibition



Example of E3 TR-FRET assay and inhibitor dose response curve.
Protein X was used as a substrate of this E3 ligase. After initial TR-FRET high throughput screening, selected candidates were used to analyze IC50 by titration assay.

Assays for Cellular Ubiquitination Activity

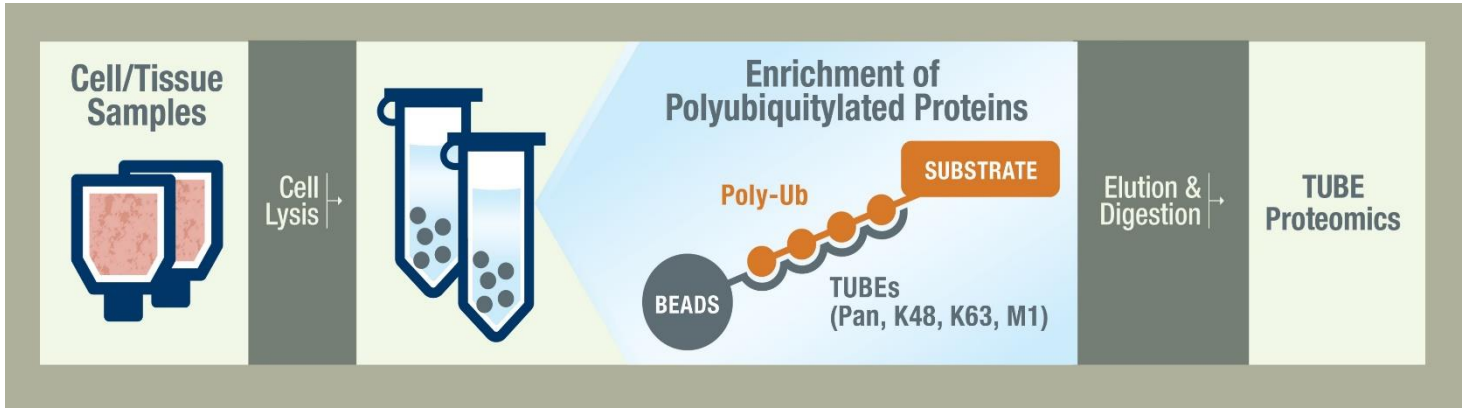


TUBE-Based Ubiquitin Mass Spec Proteomics

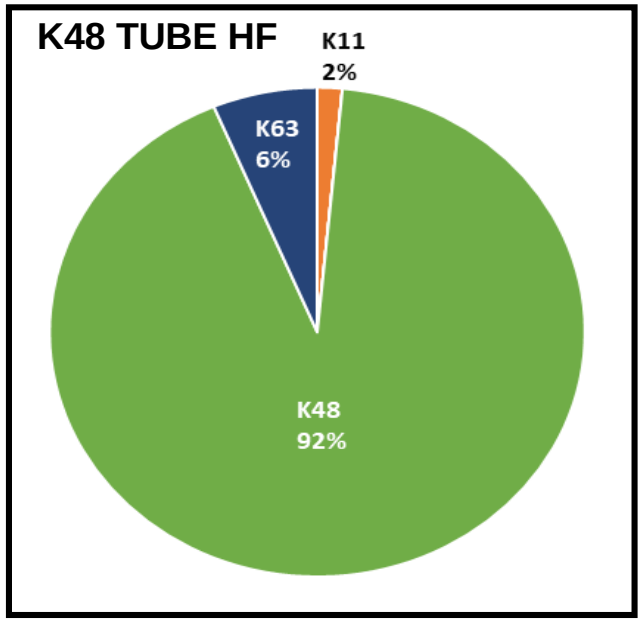
- **TUBE applications has simplified ubiquitin proteomics**
- **Rapid and quantitative analysis of biomarkers from cell and tissue**
- **Quantitative method for examining drug effect of PROTAC in cells**
- **Inexpensive and simple, no need for SILAC or other labeling protocols**

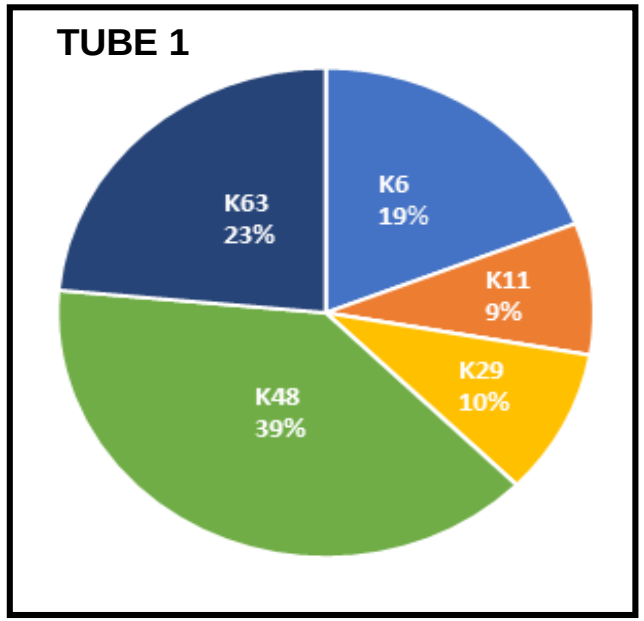
Ubiquitin Proteomics bypassing SILAC

Workflow



Experimental Results





Remarkable selectivity of K48 TUBE HF for K48-linked ubiquitinated substrates in neuronal cells.

LifeSensors Drug Discovery Platform

- ~35 biologically active DUBs and ~30 active E3 ligases
- Developed ~25 DUB assays, ~10 E3 ligase and PROTAC assays for HTS
- Ability to screen >500,000 compounds
- Enzyme selectivity panels and compound profiling
- Cellular biomarkers and target tissue PD markers
- TUBE applications

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Contact Us!

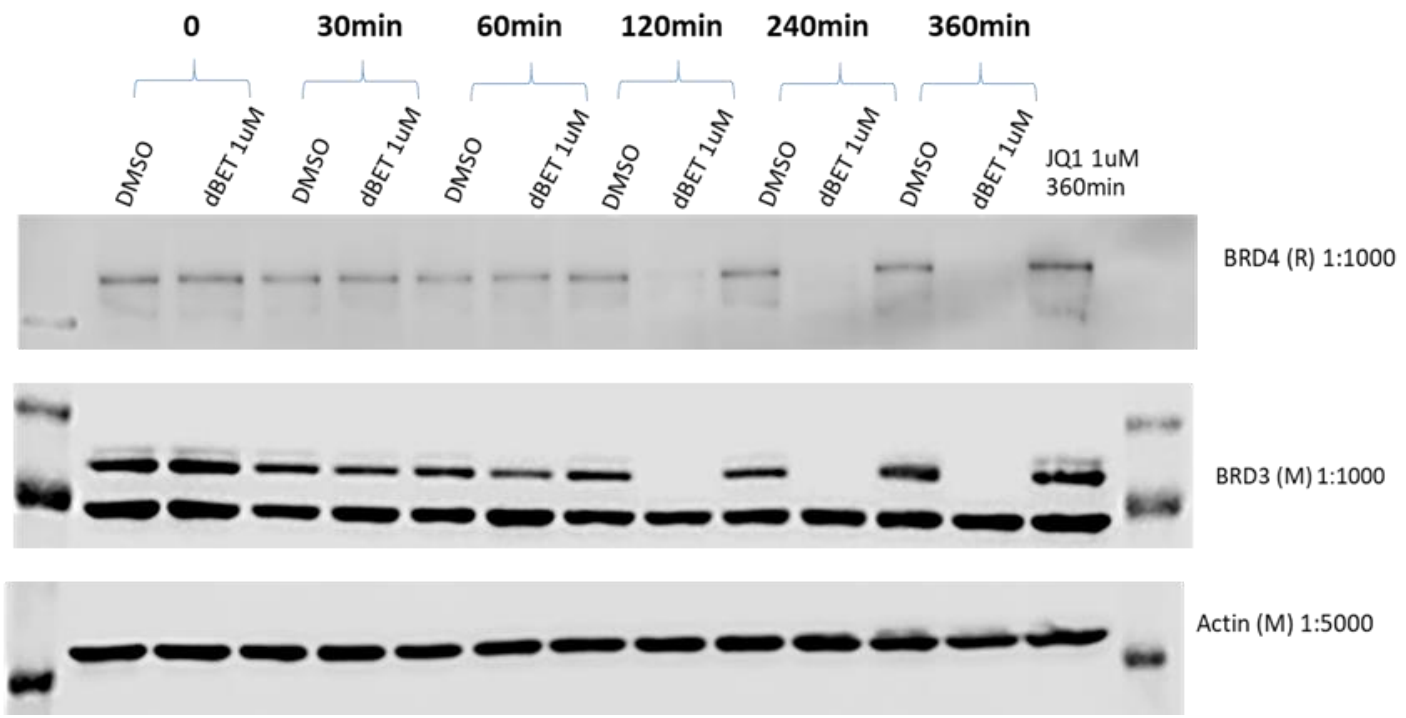
We are your partner for PROTAC drug discovery

Contact Information:

Research & Product Inquiries	R&D	info@lifesensors.com	610-644-8845 (339)
Custom Assays Applications	BD	bd@lifesensors.com	610-644-8845 (304)

BRD3 and BRD4 Cereblon PROTAC Time Course

HEK 293T



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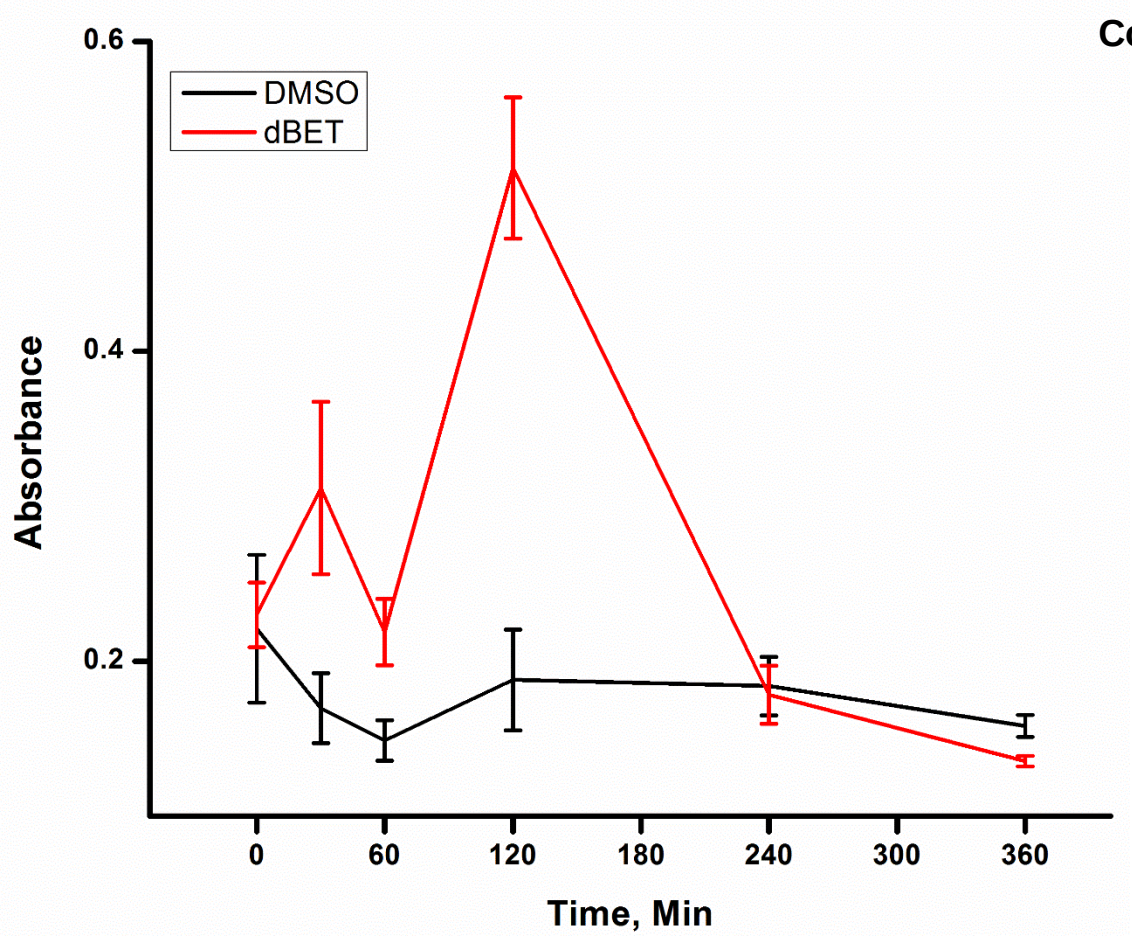
Hek293T



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