Establishing Ubiquitylation Patterns in Cells: Efficient Monitoring of Oncogenic Ubiquitylation Activity

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LifeSensors

Introduction

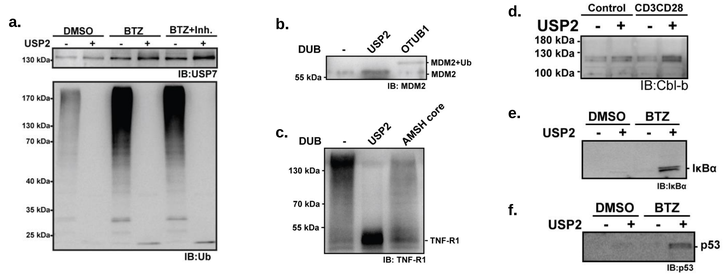
Ubiquitylation is a versatile post-translational modification involved in nearly all biological functions in eukaryotes. Ubiquitylation is catalyzed by three enzymes: ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2) and ubiquitin ligase (E3). The diverse topology and chain length of ubiquitylation provides unique ubiquitin codes, which lead to different cellular outcomes. Furthermore, ubiquitin chains can be removed by deubiquitylating enzymes (DUBs). Protein ubiquitylation has emerged as a key regulatory mechanism of protein targets in oncology and immunooncology.

Detection and isolation of ubiquitylation on target proteins is critical for understanding the myriad functions of ubiquitylation in cancer and other diseases. To date, the only effective way to measure these effects has been by immunoprecipitation and immunoblot analyses (IP/IB) or by mass spectroscopic methods. These methods are either insensitive or low throughput and at best, only semi-quantitative. Here, we have developed three assays to efficiently capture and quantify the ubiquitylation signal:

- **UbiTest**
  An immunoblot based assay to determine target protein ubiquitylation status, including chain linkages.
- **High Throughput (HT)-UbiTest**
  A high-throughput assay that can determine target protein ubiquitylation status in multiple cell samples.
- **UbiQuant S**
  A cellular assay to quantify target protein ubiquitylation, which is adaptable to ELISA and AlphaLISA formats.

Abbreviations
TUBE: *Tandem Ubiquitin Binding Entity* ***POI:*** *protein of interest* ***BTZ:*** *bortezomib (proteasome inhibitor)* ***DUB:*** *Deubiquitylation enzyme*

POI Ubiquitylation by UbiTest



a. Determine USP7 ubiquitylation in Jurkat cells after compound treatment.
b. Determine MDM2 ubiquitylation status and linkage in Jurkat cell treated with BTZ. OTUB1 is a K48-specific DUB.
c. Determine TNF-R1 ubiquitylation status and linkage in Jurkat cell treated with TNFα. AMSH is a K63-specific DUB.
d. Determine Cbl-b ubiquitylation in human T cells after TCR stimulation.
e. Determine IκBα ubiquitylation in Jurkat cells after BTZ treatment.
f. Determine p53 ubiquitylation in MM. 1S cells after BTZ treatment.

p53 HT-UbiTest Assay Validation

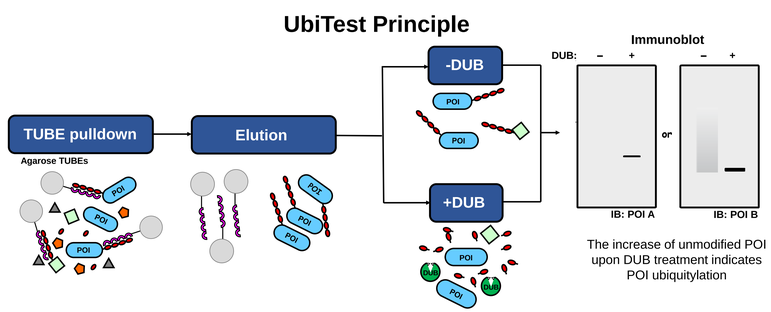
a. Determination of p53 ubiquitylation in MM.1S cells using HT-UbiTest
b. Validation of p53 HT-UbiTest assay using immunoblot-based UbiTest
c. Quantification of p53 UbiTest immunoblot

LifeSensors HT-UbiTest assays

Target	Detection method	Req #
p53	AlphaLISA	HU 001
LAT	AlphaLISA	HU 002
Zap70	TR-FRET	HU 003

UbiTest Immunoblot Assay

UbiTest Principle

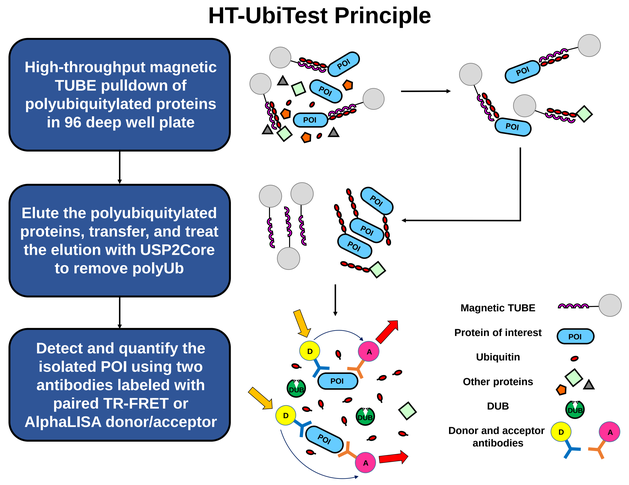


UbiTest vs. Traditional Ubiquitin IP/IB

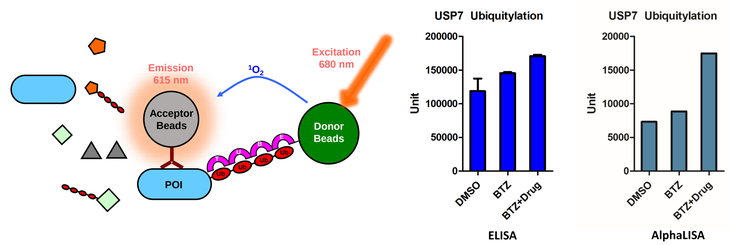
UbiTest (Catalog # UM411)	**Traditional Ubiquitin IP/IB**
➢ TUBE and DUB confirm ubiquitylation.	➢ The smeared signal may also indicate other modifications such as SUMOylation, neddylation and phosphorylation.
➢ Ubiquitylated proteins are enriched by TUBE pulldown.	➢ The smear may be very light or undetectable due to a dilutive effect.
➢ DUB digestion removes all the ubiquitin, exposing the epitopes.	➢ Antibody target epitopes may be blocked by ubiquitin modification on the target protein.

High Throughput (HT)-UbiTest

HT-UbiTest Principle



UbiQuant S Concept and USP7 Example



Conclusions

- **UbiTest, HT-UbiTest, and UbiQuant S are efficient tools to study protein ubiquitylation in cell samples.**
- **These assays can be used for detecting ubiquitylation of endogenous substrates.**
- **UbiTest is a straightforward and sensitive method to determine substrate ubiquitylation status.**
- **HT-UbiTest and UbiQuant S provide high-throughput platforms for monitoring the dynamics of target ubiquitylation in response to drug treatment and other stimuli.**