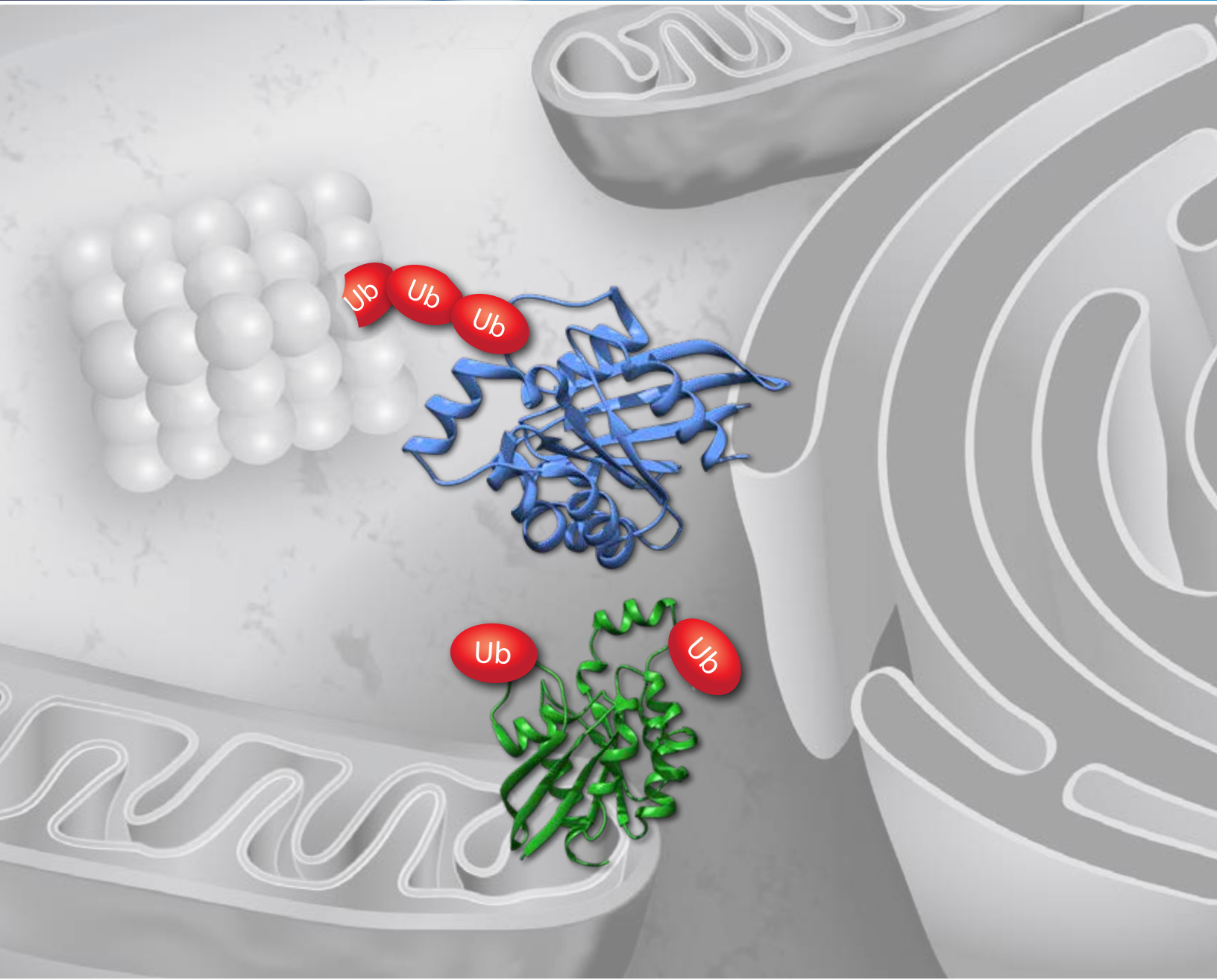
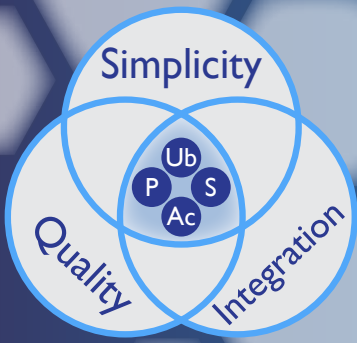


Maximizing Ubiquitin Discovery

The first mono- and poly-ubiquitin
UBD detection system



About Ubiquitin Protein Modifications

Poly-ubiquitination of a target protein is a well established mechanism to regulate proteasome mediated degradation, and mis-regulation of this essential post-translational modification (PTM) has been shown to promote disease progression. It is now appreciated that both poly- and mono-ubiquitination can control critical protein functions like protein:protein interaction, spatial regulation, and crosstalk with other PTMs (1,2). Proteome studies suggest that thousands of proteins are regulated by ubiquitin (3); however, tools to study endogenous ubiquitination of a target protein are not readily available. Many studies investigating target protein ubiquitination are performed using Ub and protein overexpression systems. While these data are clearly important, and can provide critical information such as site specificity, Ub overexpression can also mask physiologic changes (4,5). To complement existing tools, and to allow non-Ub specialists to quickly determine if their target protein is endogenously ubiquitinated, Cytoskeleton developed a ubiquitination detection kit that will efficiently determine whether a target protein is mono- and/or poly- ubiquitinated under physiologic conditions.

The first UBD-Based Mono- And Poly-Ubiquitin Enrichment Affinity Beads

Affinity Bead Test: UBA01 compared to FK2

Affinity Bead Test: UBA01 vs other UBDs

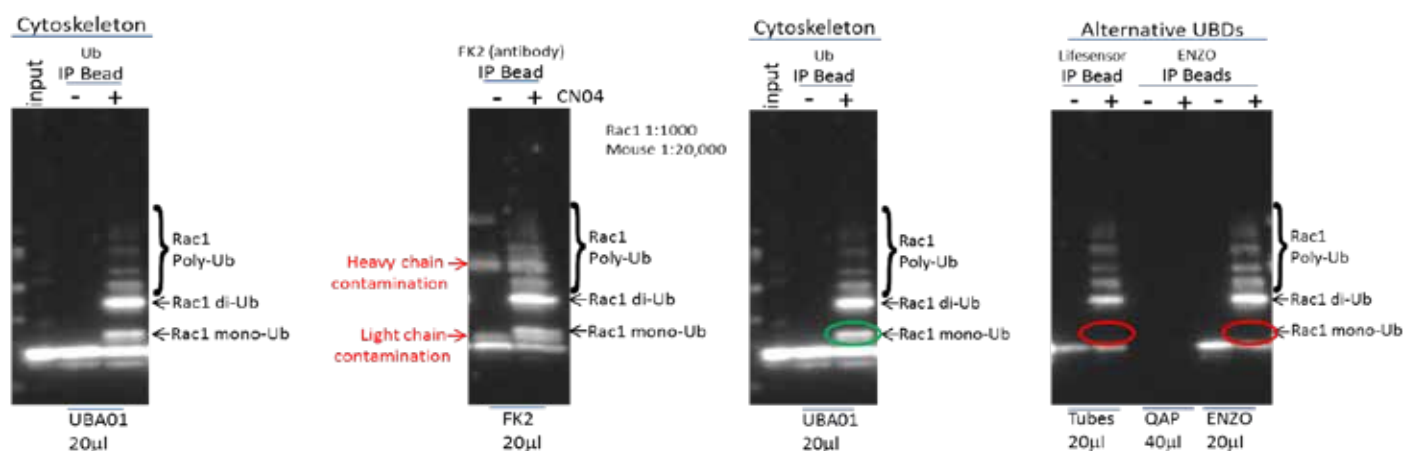


Figure 1 Legend: (A) 3T3 cells pretreated with MG-132 and +/- cytosolic necrotizing factor 1 (CN04) were lysed with BlastR lysis buffer (Cytoskeleton Inc.). 300µg of lysate was incubated with the specified amount of UBA01 (Cytoskeleton Inc, ubiquitination affinity beads), FK2 (MBL Life science, anti-multi ubiquitin mAb-agarose, D058-8), Tubes (Lifesensors UM401, agarose-Tube 1), QAP (ENZO, UBIQAPTURE-Q kit, BML-UW8995-0001), and ENZO (ENZO, DSK2 UBA domain, BML-UW9835-0500) ubiquitin affinity reagents. Immunoprecipitated samples were separated by SDS-PAGE and transferred to PVDF. Western blot was performed with a Rac1 antibody.

Ubiquitin Products

Description	Amount	Item #
Signal-Seeker™ Ubiquitination Detection Kit	30 assays	BK161
Signal-Seeker™ Ubiquitination Detection Kit	10 assays	BK161-S
Ubiquitination Affinity Beads	40 assays	UBA01-beads
Ubiquitination Control Beads	10 assays	CUB02-beads
Ubiquitin Mouse Antibody (P4D1)	2 x 100 µl	AUB01
Ubiquitin Mouse Antibody-HRP labeled	1 x 100 µl	AUB01-HRP

References

1. Yau R, Rape M. The increasing complexity of the ubiquitin code. *Nat Cell Biol.* 2016;18(6):579-86, 10.1038/ncb3358.
2. Swatek KN, Komander D. Ubiquitin modifications. *Cell Res.* 2016;26(4):399-422, 10.1038/cr.2016.39.
3. Akimov V, Olsen LCB, Hansen SVF, Barrio-Hernandez I, Puglia M, Jensen SS, et al. StUbEX PLUS-A Modified Stable Tagged Ubiquitin Exchange System for Peptide Level Purification and In-Depth Mapping of Ubiquitination Sites. *J Proteome Res.* 2018;17(1):296-304, 10.1021/acs.jproteome.7b00566.
4. Emmerich CH, Cohen P. Optimising methods for the preservation, capture and identification of ubiquitin chains and ubiquitylated proteins by immunoblotting. *Biochem Biophys Res Commun.* 2015;466(1):1-14, 10.1016/j.bbrc.2015.08.109.
5. Horita H, Law A, Hong S, Middleton K. A simple toolset to identify endogenous post-translational modifications for a target protein: a snapshot of the EGFR signaling pathway. *Biosci Rep.* 2017, 10.1042/BSR20170919.

Superior Tools Produce Exceptional Endogenous Ubiquitination Detection

Robust Enrichment Compared to FK2

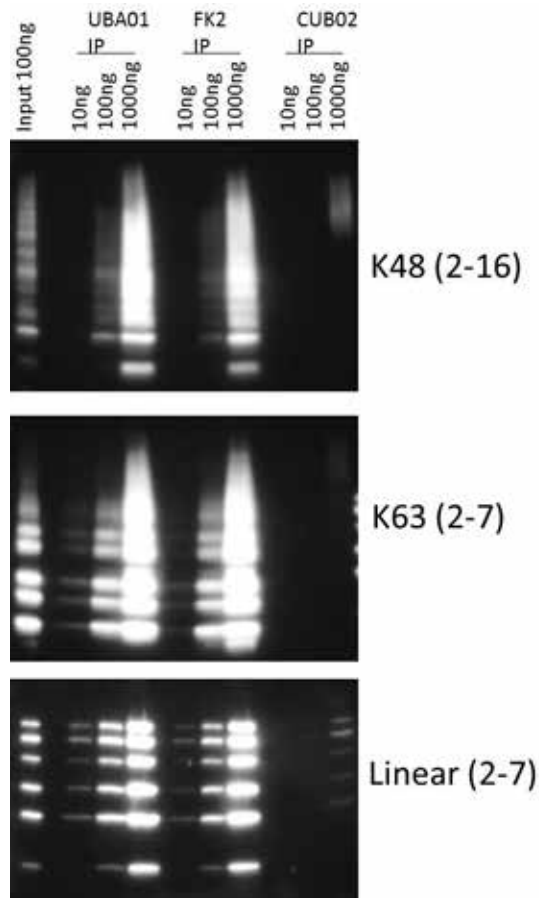


Figure 2 Legend: K48, K63, and linear ubiquitin chains were reconstituted as recommended. 10, 100, and 1000ng of each ubiquitin chain were incubated with ubiquitin affinity beads (UBA01), control beads (CUB02), and ubiquitin antibody (FK2) according to the IP method. Immuno-precipitated samples were separated by SDS-PAGE and transferred to PVDF. Western blot was performed with an anti-ubiquitin antibody (AUB01-HRP) to measure chain-specific ubiquitin isolation.

Endogenous Ub Detection of a Target Protein

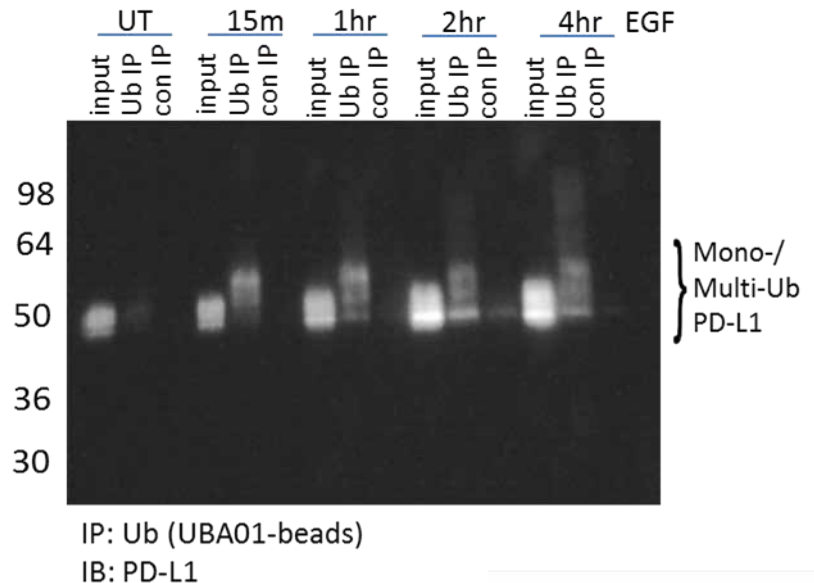


Figure 3 Legend: Serum-restricted A431 cells were stimulated with EGF for the given time period. Cells were lysed with BlastR lysis buffer. 1 mg of lysate from each timepoint was incubated with ubiquitin affinity beads (UBA01) or control beads (CUB02) to capture endogenous ubiquitinated proteins. WCL and immunoprecipitated samples were separated by SDS-PAGE and analyzed by western blot for PD-L1. Adapted from Horita et al. Neoplasia 2017.

Ub Immunofluorescence

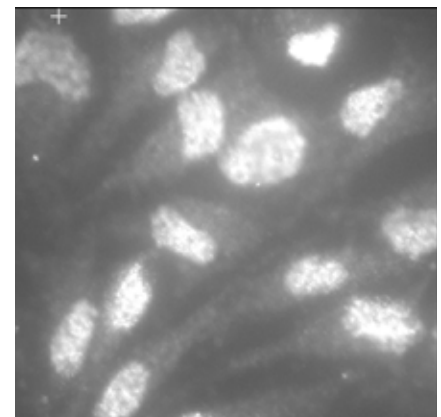
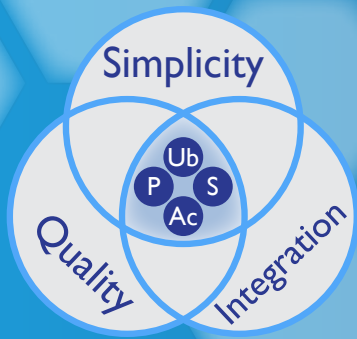


Figure 4 Legend: HeLa cells were stained and visualized by fluorescence microscopy as described in the datasheet. Ubiquitin targeted cytoplasmic and nuclear proteins and free ubiquitin were stained using AUB01 at 1:500 dilution.

Applications

Application	Product	Validation Data
Western Blot	Ubiquitin Mouse Antibody-HRP labeled, Cat. # AUB01-HRP	Yes
	Ubiquitin Mouse Antibody (P4D1), Cat. # AUB01	Yes
Immunofluorescence	Ubiquitin Mouse Antibody (P4D1), Cat. # AUB01	Yes
Immunoprecipitation	Signal-Seeker™ Ubiquitination Detection Kit, Cat. # BK161	Yes
	Signal-Seeker™ Ubiquitination Detection Kit, Cat. # BK161-S	Yes
	Ubiquitination Affinity Beads, Cat. # UBA01-beads	Yes

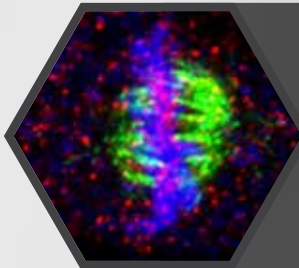
*Recommended products for each application are highlighted in blue



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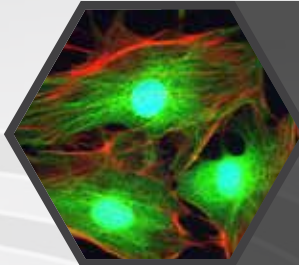
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Signal-Seeker™ SUMOylation 2/3 Tools

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