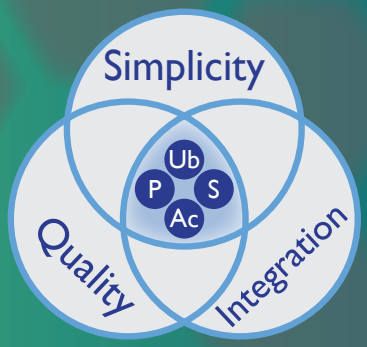
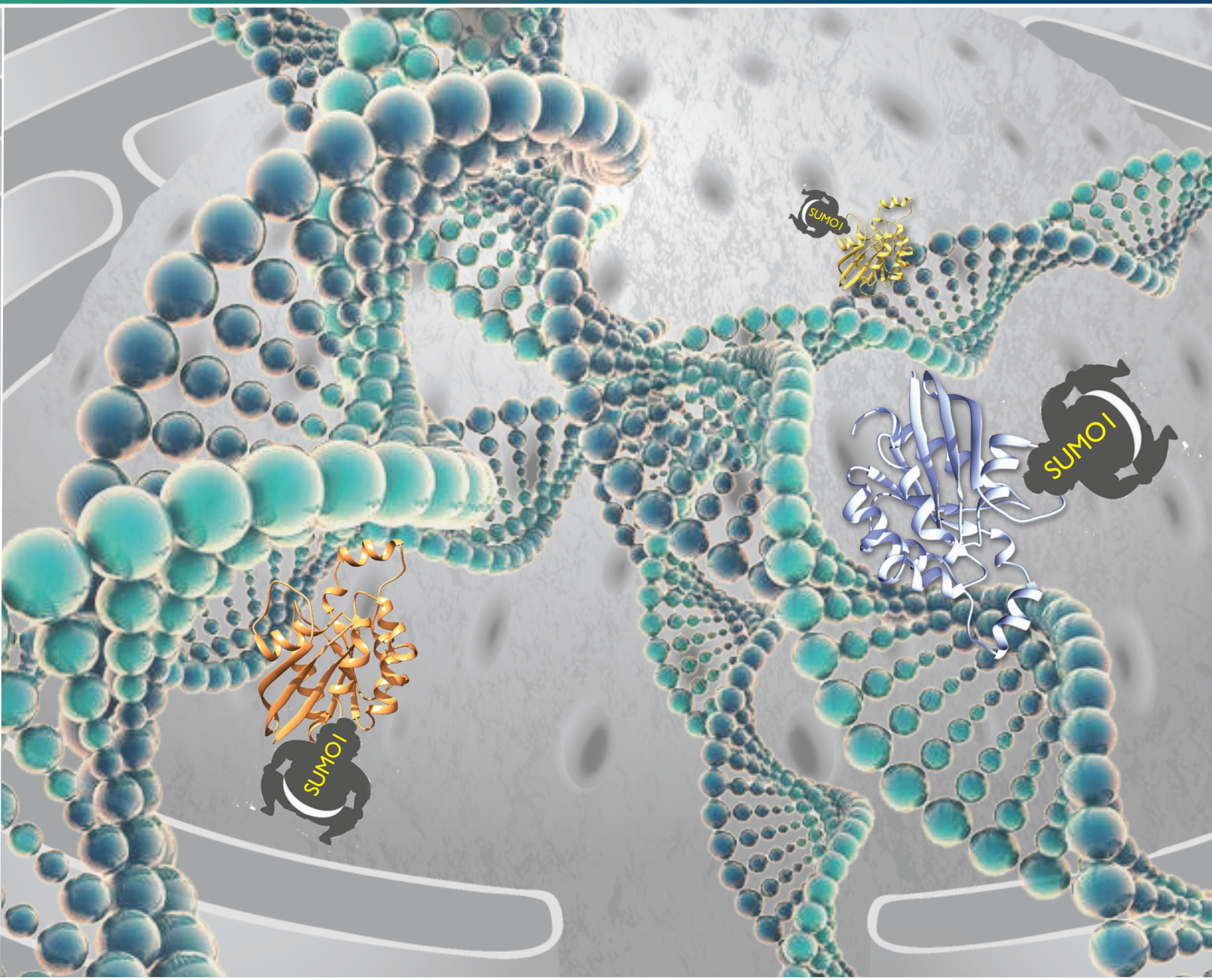


Advancing SUMO1 Discovery

The next generation of tools for endogenous SUMO1 detection



The Protein Experts

Cytoskeleton, Inc.

Signal-Seeker Toolkits
by ***Cytoskeleton, Inc.***

Signal-Seeker™ SUMO1 Tools

About SUMOylation1 Protein Modifications

In mammalian cells, the small ubiquitin-like modifier (SUMO) family contains four isoforms (SUMO1, SUMO2, SUMO3, and SUMO4), and functions as a 12 kDa posttranslational modification (PTM) protein. SUMO2 and SUMO3 are nearly identical, differing in only three amino acid residues. SUMO1, also known as SMT3C, Sentrin, GMP1, UBL1, and PIC1, shares 48% identity with SUMO2/3 (1). SUMO4 is about 85% identical to SUMO2/3, but it is unclear whether SUMO4 can be conjugated to substrates (2). Proteins are post-translationally modified by SUMO conjugation (SUMOylation) to an acceptor lysine residue within a target protein consensus sequence ψKXE (where ψ represents a hydrophobic amino acid and X represents any amino acid). Similar to ubiquitination, SUMOylation requires a three enzymes system (E1, E2, and E3) to conjugate SUMO covalently to target substrates. The covalently linked SUMO can be removed by sentrin-specific proteases (SENPs), a process known as deSUMOylation (3). SUMOylation is a highly dynamic, reversible PTM that regulates the activity, subcellular localization, stability, and functions of target proteins and thereby modulates almost all major cellular pathways (4). As SUMO modifications are relatively new compared to other PTMs like ubiquitination or acetylation, tools to investigate SUMO are not well established. Appropriate lysis buffers, inhibitors, and high-quality affinity antibodies and beads are all critical reagents for effectively studying PTMs; in particular, SUMO modifications. Here we introduce Cytoskeleton's highly validated SUMO 1 antibody and affinity beads, as well as the first comprehensive kit for endogenous SUMO1 detection (Cat. # BK165).

Robust Antibody Detection

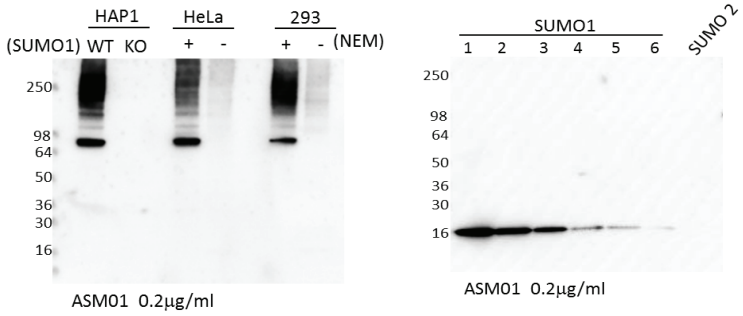


Figure 1 Legend: ASM01 was used at a 1:5000 dilution (0.2 mg/mL) following the recommended western blot protocol (see above). Figure 1A: HAP1 wildtype (WT) or SUMO1 knockout (KO) lysate, HeLa cell +/- NEM lysate and 293 cell +/- NEM lysate was obtained using BlastR lysis and filter system. 10 mg of each lysate were separated by SDS-PAGE and transferred to PVDF. Robust SUMO1 profiles were detected for every cell type. Specificity is shown by the lack of SUMO1 detection in SUMO1 KO cells and significantly diminished profiles in lysates prepared in the absence of the SENP inhibitor (NEM). Figure 1B: Titration of recombinant SUMO1 lanes 1-6 contain 5.0, 2.5, 1.25, 0.6, 0.3, and 0.15 ng SUMO1, while lane 7 contains 1000 imaged simultaneously to ensure identical experimental conditions.

Outperforming the Competition

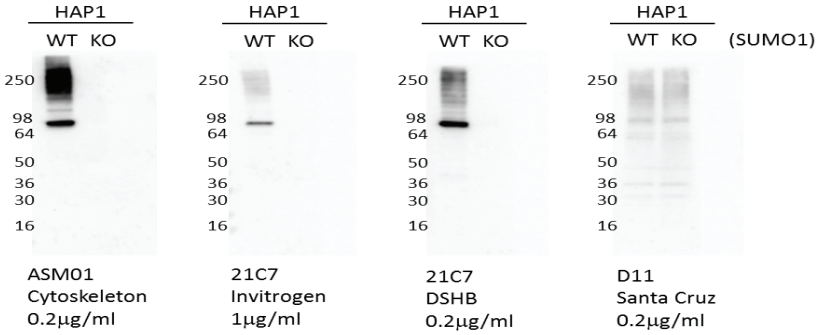


Figure 2 Legend: HAP1 WT and KO lysate as prepared in 1A was used. 10 mg of each lysate were separated by SDS-PAGE and transferred to PVDF. SUMO1 proteins were detected using the recommended concentrations of ASM01 (Cytoskeleton), 21C7 (Invitrogen—purified), 21C7 (DSHB—supernatant), and D11 (Santa Cruz) as shown in the figure. All samples were developed and imaged simultaneously to ensure identical experimental conditions.

SUMOylation 2/3 Products

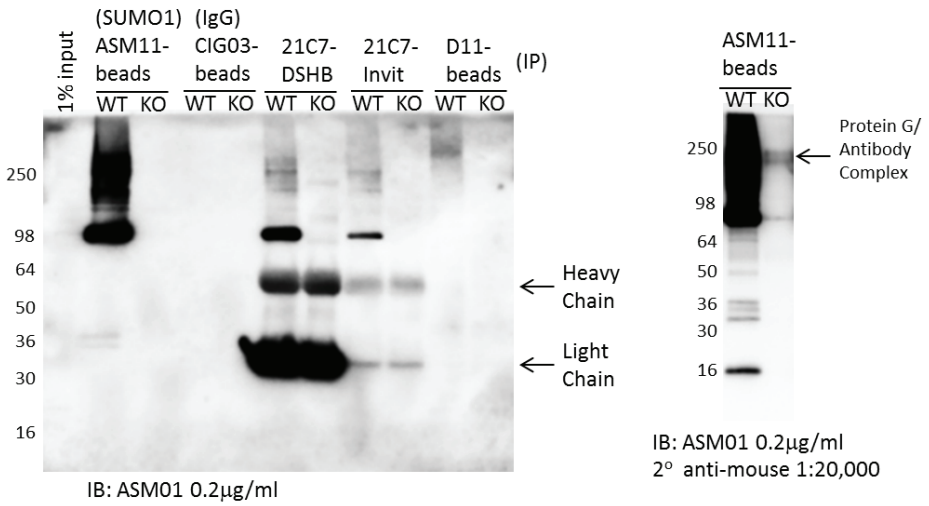
Description	Amount	Item #
Signal-Seeker™ SUMOylation 1 Detection Kit	30 assays	BK165
Signal-Seeker™ SUMOylation 1 Detection Kit	10 assays	BK165-S
SUMOylation 1 Affinity Beads	30 assays	ASM11-beads
SUMO1 IP Control Beads	10 assays	CIG03-beads
SUMO1 Mouse Antibody (5D8B16)	1 x 100 µl	ASM01

References

1. Guo C. and Henley J. 2014. Wrestling with stress: Roles of protein SUMOylation and deSUMOylation in cell stress response. IUBMB Life. 66, 71-77.
2. Owerbach D. et al. 2005. A proline-90 residue unique to SUMO-4 prevents maturation and sumoylation. Biochem. Biophys. Res. Comm. 337, 517-520.
3. Yang W. and Paschen W. 2015. SUMO proteomics to decipher the SUMO-modified proteome regulated by various diseases. Proteomics. 15, 1181-1191.
4. 4. Kira B. et al. 2012. SUMOylation in carcinogenesis. Cancer Lett. 316, 113-125.

Simplifying and Enhancing SUMO1 Detection

SUMO1 Affinity Beads and Kits



Essential Kit Components

- Universal lysis system
- protein quantitation system
- IP Affinity Beads
- Control Beads
- Key Inhibitors
- Wash reagents
- Elution reagents
- chemiluminescence

Figure 4 Legend: Total SUMO1 profiles. (A) HAP1 wildtype (WT) or SUMO1 knockout (KO) lysate, was obtained using BlastR lysis and filter system. 1 mg of each lysate were incubated with 40 mg of each SUMO1 affinity reagent: ASM11-beads (Cytoskeleton), 21C7 (Invitrogen—purified), 21C7 (DSHB—supernatant), D11-beads (Santa Cruz) and conjugated SUMO1 IgG control beads (CIG03). 21C7 antibodies were captured with protein G agarose beads to enrich for SUMO-1 modified proteins. Samples were separated by SDS-PAGE and transferred to PVDF. Enriched SUMO1 samples were analyzed by western blot using ASM01 (Cytoskeleton) antibody at 1:5000, and mouse Trubelot Ultra-HRP secondary at 1:1000 in 5% milk. Trueblot secondary was used to minimize heavy and light chain detection from 21C7 samples. (B): IP was performed using ASM11 as in Fig 1A. SUMO1 modified proteins were visualized with ASM01 1:5000, and anti-mouse secondary at 1:20,000 to highlight the profile of SUMOylated proteins in the region between 64-30 kDa that may be masked by heavy and light chain interference when using unconjugated antibodies for IP.

Investigate Endogenous Target Protein SUMO1 Modifications With Confidence

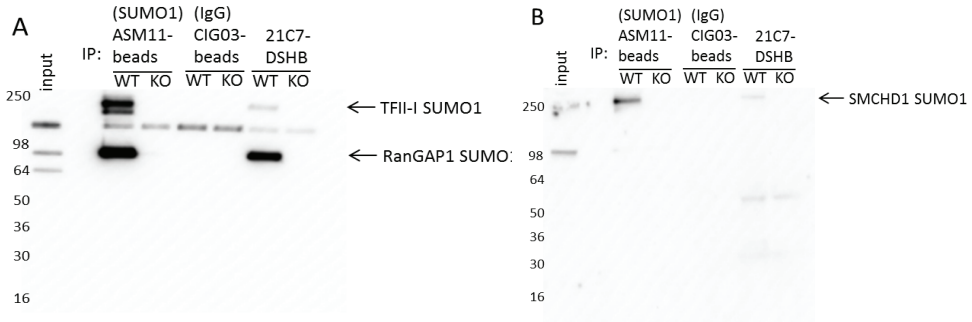
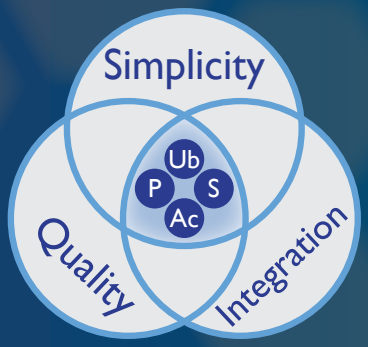


Figure 5 Legend: SUMO1 modified target proteins. HAP1 wildtype (WT) or SUMO1 knockout (KO) lysate, was obtained using BlastR lysis and filter system. 1 mg of each lysate was incubated with 40 µg of each SUMO1 affinity reagent: ASM11-beads (Cytoskeleton), 21C7 (DSHB—supernatant), and conjugated SUMO1 IgG control beads (CIG03). 21C7 antibodies were captured with protein G agarose beads to enrich for SUMO-1 modified proteins. Samples were separated by SDS-PAGE and transferred to PVDF. Target proteins: (A) TFII-I, RanGAP1, and (B) schmd1 were analyzed for their SUMO1 modified forms by western blot. Anti-rabbit-HRP labeled secondary antibody was used at 1:10,000.

Applications

Application	Product	Validation Data
Western Blot	SUMO1 Mouse Antibody-(5D8B16) Cat. # ASM01	Yes
Immunofluoresence	Not Tested	Yes
Immunoprecipitation	Signal-Seeker™ SUMO1 Detection Kit, Cat. # BK165	Yes
	Signal-Seeker™ SUMO1 Detection Kit, Cat. # BK165-S	Yes
	SUMOylation 1 Affinity Beads, Cat. # ASM11-beads	Yes
	SUMO1 Mouse Antibody (5D8B16), Cat. # ASM01	Yes

*Recommended products for each application are highlighted in **blue**



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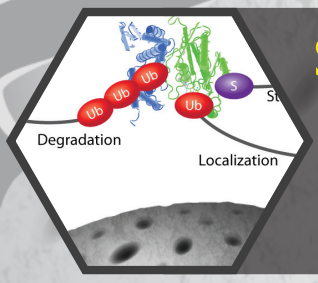
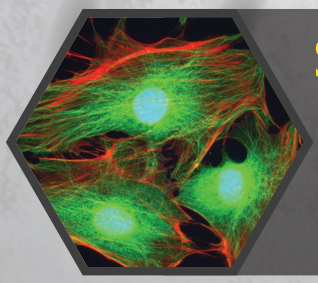


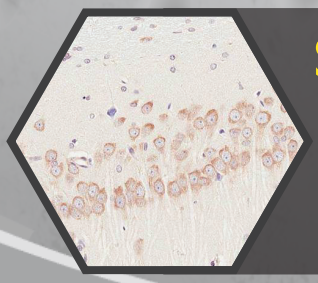
Signal-Seeker™ Ubiquitin Tools

- Mono-/Poly-Ubiquitination Detection Kit
- Mono-/Poly-Ubiquitination Affinity Beads
- Ubiquitin Antibody-HRP labeled



Signal-Seeker™ Acetyl-lysine Tools

- Acetyl-lysine Detection Kit
- Acetyl-lysine Affinity Beads
- Acetyl-lysine IF and HRP labeled Antibodies



Signal-Seeker™ Phosphotyrosine Tools

- Phosphotyrosine Detection Kit
- Phosphotyrosine Affinity Beads
- Phosphotyrosine IF and HRP labeled Antibodies

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