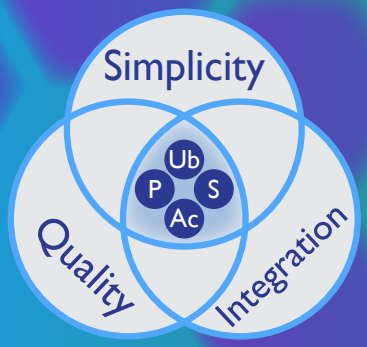


Enhancing SUMO 2/3 Discovery

The first comprehensive kit for endogenous SUMO 2/3 detection

The Protein Experts
Cytoskeleton, Inc.

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

Signal-Seeker™ SUMO 2/3 Tools

About SUMOylation 2/3 Protein Modifications

Dynamic post translational modifications (PTMs) like small ubiquitin-related modifier (SUMO)ylation provide proteins with vastly expanded functionality. SUMO modifications regulate many biological process, such as, transcriptional regulation and DNA repair (1). In vertebrates 4 paralogs of SUMO exist, SUMO-1, SUMO-2, SUMO-3, and SUMO-4 (2). SUMO-2 and SUMO-3 are referred to as SUMO 2/3 because of their high similarity, and the inability to distinguish the two molecules endogenously. Of interest, SUMO-2/3 appears to be more abundant than SUMO-1 and there is a free pool of SUMO-2/3 molecules that are utilized in response to cellular stresses (3). Recent proteomic studies have identified hundreds of proteins that are SUMOylated (4,5). Still, these data need complementary validation and mechanistic studies to better define the importance of a given protein's SUMO state both physiologically and pathologically.

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 2/3 antibodies and affinity beads (See Validation of SUMO-2/3 Antibodies White Paper: www.cytoskeleton.com/about-signal-seeker-validation-data), as well as the first comprehensive kit for endogenous SUMO 2/3 detection (Cat. # BK162).

Robust Antibody Detection

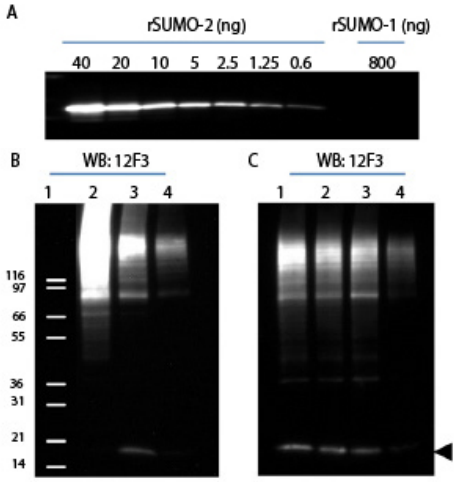


Figure 1 Legend: 12F3 SUMO 2/3 antibody (ASM23) was examined by western blot (1:500 dilution) using the following samples. (A) Titration of recombinant SUMO-2 (40-0.6 ng) and SUMO-1 (800 ng). (B) Untreated (lane 3), heat shocked (lane 2), and SUMO 2 shRNA knockdown (lane 4) Hela cell lysate was analyzed. 20µg of HeLa cell lysates were used for each sample. (C) Parental HeLa cell lysate (lane 1), SUMO-2 shRNA control lysate (lane 2), SUMO-1 shRNA knock-down cell lysate (lane 3), and SUMO-2 shRNA knock-down cell lysate (lane 4) were examine. Arrow head indicates free SUMO-2/3. SUMO 2/3 antibody is specific for SUMO 2/3 and not SUMO-1.

Superior Affinity Bead Enrichment

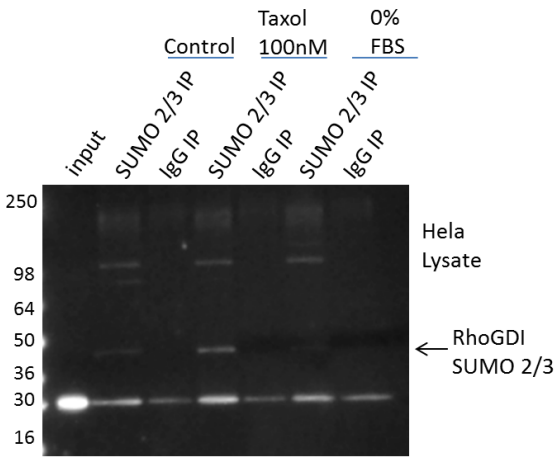


Figure 2 Legend: Untreated, Taxol 100nM, or serum restricted (0% FBS) treated Hela cells were lysed with BlastR lysis buffer (Cytoskeleton Inc.) supplemented with deSUMOylase inhibitor (NEM). 1mg of each lysate was incubated with the recommended amount of SUMO 2/3 affinity beads (ASM24-beads) or IgG control beads (CIG01) (Cytoskeleton Inc.). Immunoprecipitated samples were separated by SDS-PAGE and transferred to PVDF. Western blot was performed with RhoGDI.

SUMOylation 2/3 Products

Description	Amount	Item #
Signal-Seeker™ SUMO 2/3 Detection Kit	30 assays	BK162
Signal-Seeker™ SUMO 2/3 Detection Kit	10 assays	BK162-S
SUMO 2/3 Affinity Beads	20 assays	ASM23-beads
Mouse IgG Control Beads	10 assays	CIG01-beads
SUMO 2/3 Mouse Antibody (12F3)	2 x 100 µl	ASM23
SUMO 2/3 Mouse Antibody (11G2)	2 x 100 µl	ASM24
SUMO 2/3 Mouse Antibody-HRP labeled	1 x 100 µl	ASM23-HRP

References

1. Enserink JM. Sumo and the cellular stress response. Cell Div. 10 4, doi: 10.1186/s13008-015-0010-1 (2015).
2. Hickey CM, Wilson NR, Hochstrasser M. Function and regulation of SUMO proteases. Nat Rev Mol Cell Biol. 13 (12), 755-66, doi: 10.1038/nrm3478 (2012).
3. Saitoh H, Hinchey J. Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. J Biol Chem. 275 (9), 6252-8, doi: (2000).
4. Bruderer R, Tatham MH, Plechanovova A, Matic I, Garg AK, Hay RT. Purification and identification of endogenous polySUMO conjugates. EMBO Rep. 12 (2), 142-8, doi: 10.1038/embor.2010.206 (2011).
5. Yang W, Paschen W. SUMO proteomics to decipher the SUMO-modified proteome regulated by various diseases. Proteomics. 15 (5-6), 1181-91, doi: 10.1002/pmic.201400298 (2015).

The BK162 Kit Simplifies Endogenous SUMO 2/3 Detection

Essential Lysis System

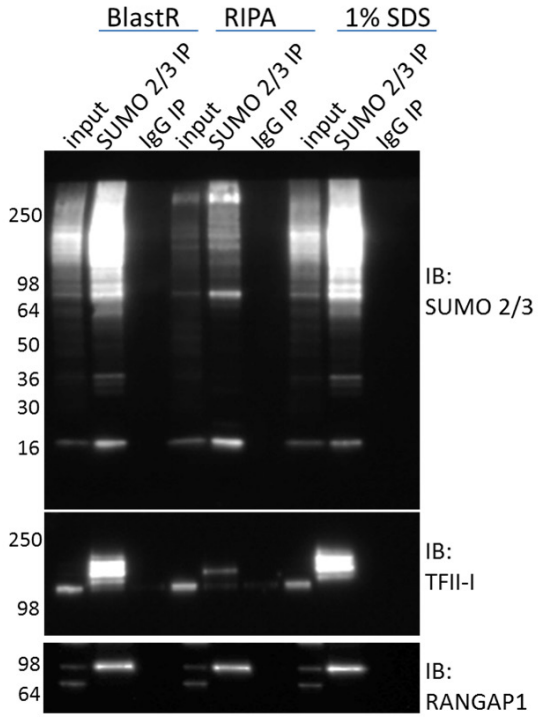


Figure 3 Untreated A431 cells were lysed with BlastR lysis buffer (Cytoskeleton Inc.), RIPA lysis buffer, or 1% SDS denaturing lysis buffer supplemented with deSUMOylase inhibitors (NEM+TPEN). 1mg of each lysate was incubated with the recommended amount of SUMO 2/3 affinity beads (ASM24-beads) or IgG control beads (CIG01) (Cytoskeleton Inc.). Immunoprecipitated samples were separated by SDS-PAGE and transferred to PVDF. Western blot was performed with SUMO 2/3 antibody (ASM23-HRP), TFII-I, and RANGAP1.

Key Inhibitors

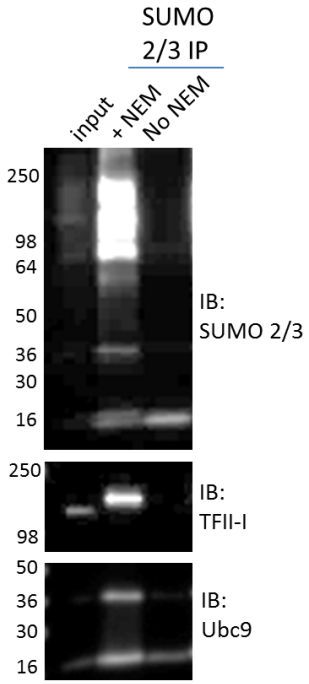


Figure 4 Legend: Untreated A431 cells were lysed with BlastR lysis buffer (Cytoskeleton Inc.) supplemented with or without deSUMOylase inhibitor (NEM). 1mg of each lysate was incubated with the recommended amount of SUMO 2/3 affinity beads (ASM24-beads) (Cytoskeleton Inc.). IP'ed samples were separated by SDS-PAGE and transferred toPVDF. Western blot was performed with SUMO 2/3 antibody (ASM23-HRP), and TFII-I.

In Vivo SUMO 2/3 Investigation

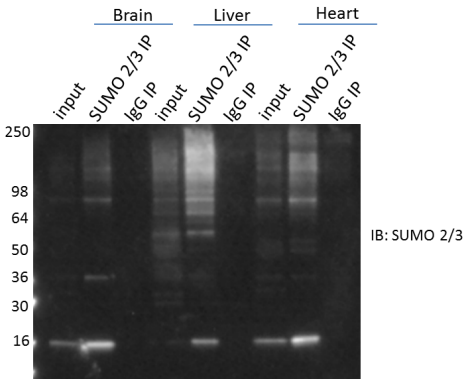
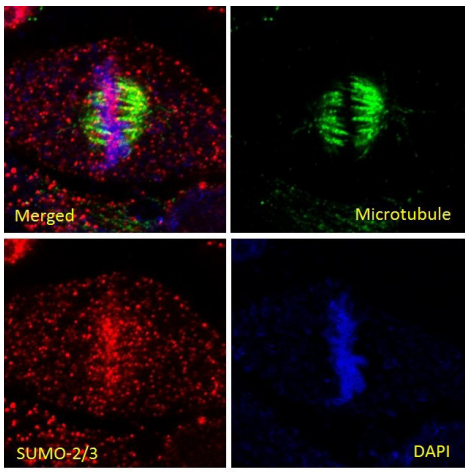


Figure 5 Legend: Mouse brain, liver, and heart tissue were lysed with BlastR lysis buffer (Cytoskeleton Inc.), supplemented with deSUMOylase inhibitor (NEM), according to the manufacturer's instructions. 1mg of each lysate was incubated with the recommended amount of SUMO 2/3 affinity beads (ASM24-beads) or IgG control beads (CIG01) (Cytoskeleton Inc.). Immunoprecipitated samples were separated by SDS-PAGE and transferred to PVDF. Western blot was performed with SUMO 2/3 HRP antibody.

SUMO 2/3 Immunofluorescence

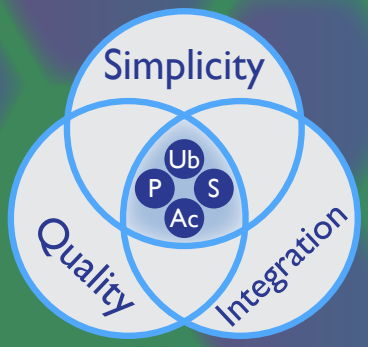


α/β-Tubulin (green): ATN02
SUMO 2/3 (Red): ASM23
DNA (Blue): DAPI
see datasheet for methodology,
www.cytoskelton.com

Applications

Application	Product	Validation Data
Western Blot	SUMO 2/3 Mouse Antibody-HRP labeled, Cat. # ASM23-HRP	Yes
	SUMO 2/3 Mouse Antibody (12F3), Cat. # ASM23	Yes
	SUMO 2/3 Mouse Antibody (11G2), Cat. # ASM24	Yes
Immunofluoresence	SUMO 2/3 Mouse Antibody (12F3), Cat. # ASM23	Yes
Immunoprecipitation	Signal-Seeker™ SUMO 2/3 Detection Kit, Cat. # BK162	Yes
	Signal-Seeker™ SUMO 2/3 Detection Kit, Cat. # BK162-S	Yes
	SUMO 2/3 Affinity Beads, Cat. # ASM24-beads	Yes
	SUMO 2/3 Mouse Antibody (12F3), Cat. # ASM23	Yes
	SUMO 2/3 Mouse Antibody (11G2), Cat. # ASM24	Yes

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



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Fax - 303.322.2257

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Denver, CO 80223, USA.

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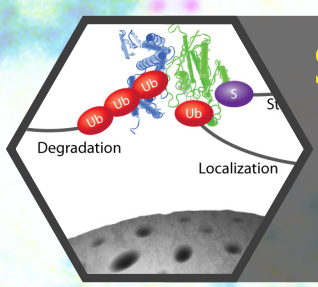
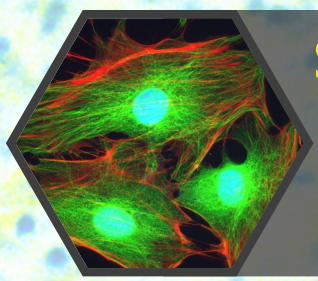
cytoskeleton.com/distributors



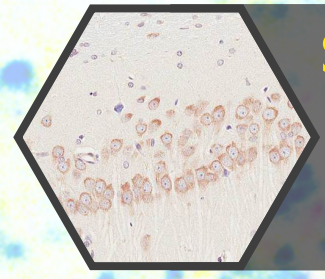
Signal-Seeker™ Ubiquitin Tools

- 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

The Protein Experts

Cytoskeleton, Inc.