V 2.0

Anti-SUMO2/3-HRP Mouse MAb

Cat. # ASM23-HRP
Lot:
Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	1 x 100 µl when reconstituted
Validated applications:	WB
Species reactivity:	Broad reactivity
Host/Isotype:	Mouse/IgG2a-kappa
Clone:	12F3

Background Information

Small Ubiquitin-related Modifiers (SUMOs) are 12 kDa post-translational modification (PTM) proteins that are highly conserved from yeast to mammalian cells[1]. In budding yeast (*Saccharomyces cerevisiae*), only one SUMO protein (Smt3) exists; in vertebrates, three major SUMO isoforms (SUMO-1, SUMO-2, and SUMO-3) are expressed in all tissue[2]. SUMO-1 is also known as SMT3C, Sentrin, GMP1, UBL1, and PIC1. Mature SUMO-2 and SUMO-3 are 97% identical in amino acid sequence (48% identity with SUMO-1) and appear to be functionally identical. SUMO proteins (SUMO-1 vs SUMO-2/3) show distinct sub-cellular localization and the expression level of SUMO-2/3 is generally higher than that of SUMO-1[3-5]. 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). While only a single SUMO-1 is ligated to target proteins, SUMO-2/3 proteins form poly-SUMO-2/3 chains on target proteins that can be terminated by SUMO-1 ligation[6,7]. SUMOylation is a highly dynamic, reversible PTM that has been demonstrated to be involved in a diverse array of cellular processes, including regulation of gene expression, protein stability, protein transport, mitosis, and protein-protein interaction[8,9].

Material

Anti-SUMO-2/3 antibody is a mouse monoclonal antibody. The antibody was raised against full-length recombinant SUMO-2 protein (Uniprot: P61956) combined with a proprietary mix of peptides that include CQIRFRFDGQPINE. The antibody has been shown to recognize a wide range of SUMO-2/3-targeted proteins in HeLa cell lysate (Fig. 1) and to detect sub-nanogram amounts of recombinant SUMO-2 (Fig. 1). Epitope mapping has identified that the antibody recognizes a sequence/structure within the peptide CQIRFRFDGQPINE. The peptide sequence is conserved in mammals, birds, and amphibians, giving the antibody broad species reactivity. ASM23 is purified by Protein G affinity chromatography and labeled with horseradish peroxidase enzyme. The antibody is supplied as a lyophilized white powder.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube.

Reconstitute each tube in 100 µl of 50% glycerol (room temperature). We do not recommend using 50% glycerol at 4°C as this can cause the lyophilized antibody to stick to the pipet tip during resuspension. Store reconstituted antibody at –20°C. Final buffer composition is 200 mM PIPES, 50% glycerol, 1% sucrose,1% dextran and 10mg/ml BSA.

The antibody can also be reconstituted in water and stored at 4°C, in this case do not freeze.

When stored and reconstituted as described, the product is stable for 6 months. **NOTE: Sodium azide is an irreversible inhibitor of HRP. Do not add sodium azide to ASM23-HRP antibody**.

Applications

Western Blot (WB) Applications

Use as indicated in method at 1:4000 dilution, sufficient for 400 ml of working strength Ab.

Figure 1: western blot application of ASM23-HRP

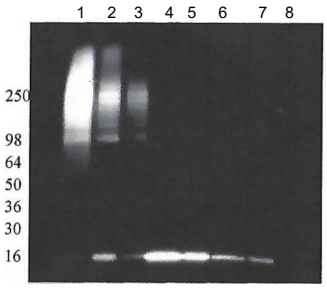

Fig 1: The following samples were run on 4 -20% SDS-PAGE; Lane 1–heat shocked 3T3 cell lysate (5 µg); Lane 2: 3T3 cell lysate (5 µg); Lane 3: 3T3 cell lysate knock-down of SUMO2/3 (5 µg); Lanes 4-7: 2 ng, 1 ng, 0.5 ng & 0.2 ng od recombinant SUMO 2 respectively; Lane 8: 1000 ng of recombinant SUMO 1. Proteins were transferred to nitrocellulose filter and western blot analysis was performed using ASM23-HRP at 1:4000 dilution in TBST/2.5% milk. Blots were developed for 30 seconds and visualized by chemiluminescence.

Western Blot Method:

1. Run protein samples and control samples on SDS-PAGE.
2. Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, 5% methanol) for 15 min at room temperature prior to electro-blotting.
3. Transfer the protein to a PVDF membrane overnight at 20V at 4°C.
4. Wash the membrane once with TBST for 10 minutes (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
5. Block the membrane surface with 5% nonfat-dry milk in TBST for 60 min at room temperature with constant agitation.
6. Incubate the membrane with a 1:4000 dilution of ASM23-HRP antibody diluted in TBST/2.5% milk for 1h at room temperature or overnight at 4°C with constant agitation.
7. Wash the membrane 6 times in TBST for 10 min each.
8. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

References

1. Machida, K. et al. (2003) Profiling the global tyrosine phosphorylation state. Mol. Cell. Proteomics 2, 215-233
2. Blagoev, B. et al. (2004) Temporal analysis of phosphotyrosine-dependent signaling networks by quantitative proteomics. Nat. Biotechnol. 22, 1139-1145
3. Schmelzle, K. et al. (2006) Temporal dynamics of tyrosine phosphorylation in insulin signaling. Diabetes 55, 2171-2179

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