**Monoclonal Antibody to SMT3 - Purified**

**Alternate names:**
SMT-3, Ubiquitin-like protein SMT3

**Quantity:**
0.1 mg

**Concentration:**
1.9 mg/ml (by UV absorbance at 280 nm)

**Background:**
Covalent modification of cellular proteins by the ubiquitin-like modifier SUMO (small ubiquitin-like modifier) regulates various cellular processes, such as nuclear transport, signal transduction, stress responses and cell cycle progression. However, in contrast to ubiquination, sumoylation does not tag proteins for degradation by the 26S proteasome but rather seems to enhance stability or modulate their subcellular compartmentalization. Ubiquitin-like proteins fall into two classes: the first class, ubiquitin-like modifiers (UBLs) function as modifiers in a manner analogous to that of ubiquitin. Examples of UBLs are SUMO, Rub1 (also called Nedd8), Apg8 and Apg12. Proteins of the second class, including parkin, RAD23 and DSK2, are designated ubiquitin-domain proteins (UDPs). These proteins contain domains that are related to ubiquitin but are otherwise unrelated to each other. In contrast to UBLs, UDPs are not conjugated to other proteins. Once covalently attached to cellular targets, SUMO regulates protein:protein and protein:DNA interactions, as well as localization and stability of the target protein. Sumoylation occurs in most eukaryotic systems, and SUMO is highly conserved from yeast to humans. Where invertebrates have only a single SUMO gene termed SMT3, three members of the SUMO family have been identified in vertebrates: SUMO-1 and the close homologues SUMO-2 and SUMO-3. SUMO has been called SMT3 (yeast), sentrin, PIC1, GMP1 and UBL1. SUMO has been shown to bind and regulate mammalian SP-RINGs (such as Mdm2, PIAS and PML), RanGAP1, RanBP2, p53, p73, HIPK2, TEL, c-Jun, Fas, Daxx, TNFR1, Topo-I, Topo-II, WRN, Sp100, IkappaB-alpha, Androgen receptor (AR), GLUT1/4, Drosophila Ttk69, Dorsal, CaMK, yeast Septins, and viral CMV-IE1/2, EBV-BZLF1, HPV/BPV-E1. These bindings implicate SUMO in the stabilization of the target proteins and/or their localization to subcellular complexes. SUMO has an apparent molecular weight of ~12 kDa, and human SUMO-1 (a 101 amino acid polypeptide) shares 50% sequence identity with SUMO-2 and SUMO-3 and with yeast SMT3. SUMO and ubiquitin only show about 18% homology, but both possess a common three-dimensional structure characterized by a tightly packed globular fold with beta-sheets wrapped around an alpha-helix.
AM08436PU-N: Monoclonal Antibody to SMT3 - Purified

**Purification:** Affinity Chromatography on protein A  
**Buffer System:** 0.02M Potassium Phosphate, 0.15M Sodium Chloride, pH 7.2  
**Preservatives:** 0.01% (w/v) Sodium Azide  
**Stabilizers:** None

**Applications:**  
- **ELISA:** 1/20,000.  
- **Western blot:** 1/500-1/2,000.  
- **Immunohistochemistry:** 1/1,000.  

Although not tested, the antibody is likely functional in Immunohistochemistry and in Immunoprecipitation. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

**Specificity:**  
This monoclonal antibody reacts with Yeast SUMO (Smt3) by western blot and ELISA. Using the specified conditions, this antibody may recognize other prominent intrinsic bands (UBLs or conjugates). Other intrinsic bands are readily detectable at lower dilutions.

**Storage:**  
Store lyophilized at 2-8°C for 6 months or at -20°C long term. After reconstitution store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C long term. Avoid repeated freezing and thawing. Shelf life: one year from despatch.

**General Readings:**  
Conjugation pathways for ubiquitin and ubiquitin-like modifiers (UBLs). Most modifiers mature by proteolytic processing from inactive precursors (a; amino acid). Arrowheads point to the cleavage sites. Ubiquitin is expressed either as polyubiquitin or as a fusion with ribosomal proteins. Conjugation requires activating (E1) and conjugating (E2) enzymes that form thiolesters (S) with the modifiers. Modification of cullins by RUB involves SCF(SKP/cullin-1/F-box protein) /CBC(cullin-2/elongin B/elonginC)-like E3 enzymes that are also involved in ubiquitination. In contrast to ubiquitin, the UBLs do not seem to form multi-UBL chains. UCRP(ISG15) resembles two ubiquitin moieties linked head-to-tail. Whether HUB1 functions as a modifier is currently unclear. APG12 and URM1 are distinct from the other modifiers because they are unrelated in sequence to ubiquitin.

Immunoblot of ySUMO fusion protein. Anti-ySUMO antibody, generated by immunization with recombinant yeast SUMO, was tested by immunoblot against a SUMO-GFP fusion protein (lane 2). While the actual molecular weight of the fusion protein is 39 kDa, the protein migrates as a 49 kDa band (arrowhead). No reactivity is seen for lane 1 which contains His-tagged GFP protein. The membrane was blocked using BLOTTO. Primary antibody was used at a 1:1,000 dilution in BLOTTO. The membrane was washed and reacted with a 1:10,000 dilution of IRDye(R) 800 Conjugated Affinity Purified Goat-anti-Mouse IgG (H&L) MX10 (800 nm channel). Molecular weight estimation was made by comparison to prestained MW markers indicated at the right (lane M, 700 nm channel). Other detection systems will yield similar results.