Certificate of Analysis

Product: **Anti-SUMO-2/3 Mouse MAb** V 1.1

Catalog #: ASM23
Product Description: Mouse monoclonal IgG2a-kappa
Clone 12F3
Purified by protein G affinity chromatography
Amount: 100 µl per tube when reconstituted (ASM23)
25 µl per tube when reconstituted (ASM23-S)
Lot: **011**

TEST	SPECIFICATON	LOT RESULTS
Appearance	White lyophilized powder	White lyophilized powder
Purity	20 µg sample shows heavy (55kD) & light chains (22kD) represent >80% of proteins by coomassie staining under denaturing conditions.	>80%
Protein Quantitation Protein quantitated with Precision Red protein assay reagent	50-150 µg per tube (ASM23) 12.5-37.5 µg per tube (ASM23-S) Aliquot size is determined by biological activity therefore µg amounts per tube will vary between lots.	100 µg 25 µg
Sensitivity/Specificity	1 ng recombinant SUMO-2 detected on western blot by chemiluminescence when 1:500 Ab dilution and 1 minute exposure time is used. A 1,000 ng sample of SUMO-1 is not detected under identical conditions.	1 ng SUMO-2 detected 1,000 ng SUMO-1 not detected
Endogenous specificity	Endogenous SUMO-2/3 detected in 20 µg of untreated HeLa cell lysate. Enhanced high molecular weight SUMOylated proteins detected upon heat shock of HeLa cells when compared to untreated cells. Reduced signal from high molecular weight proteins detected in SUMO-2 knockdown cell lysates compared to untreated cells. ASM23 used at 1:500 dilution, 1 minute exposure for chemiluminescence detection.	Endogenous SUMO-2/3 detected. Enhanced high molecular weight signal detected in heat shocked cells. Decreased signal in SUMO-2 knockdown lysates compared to untreated lysates.
Immunolocalization	Chromosomal localization (enrichment) of SUMO-2/3 during mitosis, dispersed cytoplasmic localization also visible. ASM23 was used at 1:500 dilution.	Chromosomal localization confirmed. Cytoplasmic localization confirmed.
Immunoprecipitation	Immunoprecipitation of free SUMO-2/3 and SUMOylated proteins from HeLa cell lysates. Signal strength from equivalent amounts of lysate; Heat shocked cells>untreated cells>SUMO-2 knockdown. Signals are comparable to previous Lot of ASM23.	Signal strength; heat shock>untreated>SUMO-2 knockdown. Signals comparable to previous Lot of ASM23.
QC Release Date	4/21/15	

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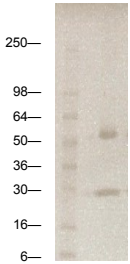
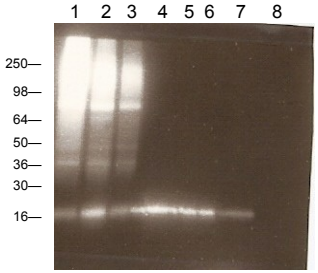
Lot specific QC Data

Purity Analysis: Lot 011



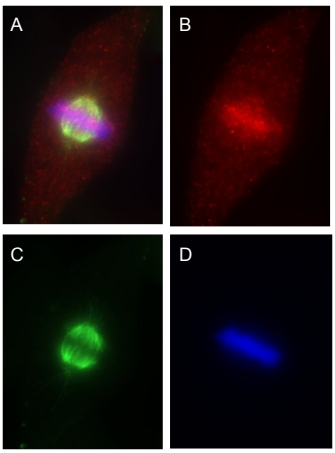
Purity analysis of protein G purified ASM23. 20 µg of ASM23 was run on 4-20% SDS -PAGE. Proteins were stained with coomassie blue. Bands at 22 kD and 55 kD represent antibody heavy and light chains respectively.

Sensitivity/Specificity: Lot 011



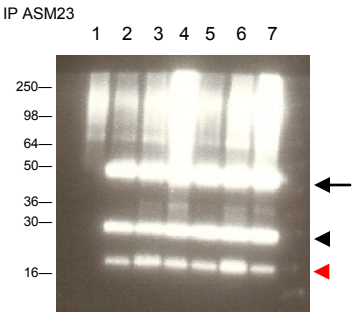
Lane 1: 20 µg lysate from heat shocked HeLa cells, Lane 2: 20 µg lysate from untreated HeLa cells, Lane 3: 20 µg lysate from SUMO-2 shRNA knockdown HeLa cells, Lane 4-7: 2 ng, 1 ng, 0.5 ng and 0.2 ng recombinant SUMO-2 respectively, Lane 8: 1000 ng recombinant SUMO-1. After SDS-PAGE and transfer to PDVF membranes, blots were probed with 1:500 dilution of ASM23 (clone 12F3) as detailed in product data sheet. NOTE: the Ab will recognize SUMO-3 in the knockdown sample.

Immunolocalization: Lot 011



HeLa cells were stained and visualized by fluorescence microscopy as described in the product data sheet for mitotic cells. The cells were stained against α/β-tubulin (sheep anti-tubulin Ab, Cat# ATN02, green) and SUMO-2/3 (12F3, red). DNA was stained with DAPI. Enrichment of SUMO 2/3 at chromosomes can be observed during mitosis.

Immunoprecipitation: Lot 011



3 mg of cell lysate from heat shocked HeLa cells (Lanes 4 & 7), untreated HeLa cells (Lanes 3 & 6) and SUMO-2 shRNA knockdown HeLa cells (Lanes 2 & 5) were incubated with 90 µl of ASM23 anti-SUMO-2/3 antibody according to the protocol in the product data sheet. Lanes 2-4 represent data using ASM23 Lot 009, Lanes 5-7 represent data from ASM23 Lot 011. Immunoprecipitated SUMO-2/3 modified proteins were run on SDS-PAGE, transferred to PVDF membranes and probed with a 1:500 dilution of ASM23. Arrow indicates heavy chain, black arrowhead indicates light chain, red arrowhead indicates free SUMO-2/3. Lane 1 is 10 µg of input lysate from heat shocked HeLa cells.