Certificate of Analysis

Product: **Anti-Acetyl Lysine Mouse MAb** V 1.0

Catalog #: AAC01
Product Description: Mouse monoclonal IgG2b
Clone 3C6.08.20
Purified by protein G affinity chromatography
Amount: 100 µl per tube when reconstituted
Lot: **013**

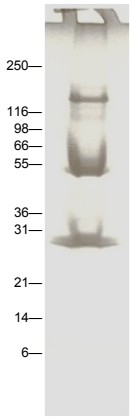
TEST	SPECIFICATON	LOT RESULTS
Appearance	White lyophilized powder	White lyophilized powder
Purity	20 µg sample shows heavy (55kD) & light chains (22kD) represent >80% of proteins by coomassie staining under denaturing conditions.	>80%
Protein Quantitation Protein quantitated with Precision Red protein assay reagent	50-150 µg per tube Aliquot size is determined by biological activity therefore µg amounts per tube will vary between lots.	110 µg
Sensitivity/Specificity	5 pg acetylated BSA (Cat# AACX1) detected on western blot by chemiluminescence when 1:500 Ab dilution and 2 minute exposure time is used. A 1,000,000 pg sample of non-acetylated BSA is not detected under identical conditions.	5pg acetylated BSA detected. 1,000,000 pg BSA not detected
Endogenous specificity	Detects Trichostatin A (TSA) enhanced tubulin (55kD) and histone (14-16kD) acetylation in 20 µg of A431 cell lysates in western blot analysis. AAC01 used at 1:500 dilution, 2 minutes exposure for chemiluminescence detection.	Acetylated tubulin and histones detected
Immunolocalization	Detects enhanced nuclear and microtubule acetylation after TSA treatment. AAC01 used at 1:200 dilution.	Nuclear and microtubule detection in TSA treated A431 cells
Immunoprecipitation	AAC01 (20 µl) bound to protein G-beads (30 µl) will enrich acetylated proteins from 1-1.5 mg of TSA treated cell lysates. Detection of acetylated histones is >4 times greater in TSA treated vs untreated cell lysate.	>4 fold enhancement of histone enrichment in TSA treated cell lysate
QC Release Date	4/18/2014	

Kim Middleton
QC Manager

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

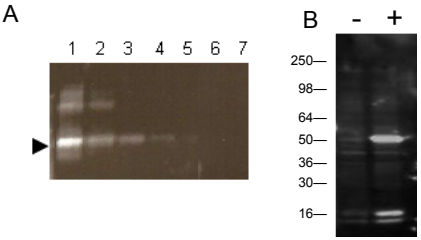
Lot specific QC Data

Purity Analysis: Lot 013



Purity analysis of protein G purified AAC01. 20 µg of AAC01 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. The band at approximately 150 kD is non-reduced antibody.

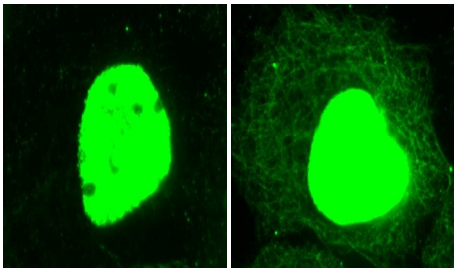
Sensitivity/Specificity: Lot 013



A) Titration of acetylated BSA lanes 1-5 contain 0.5, 0.1, 0.05, 0.01, and 0.005 ng Ac-BSA, lanes 6-7 contain 500 and 1000 ng non-acetylated BSA, respectively. AAC01 recognizes 0.005 ng of chemically acetylated BSA. Arrowhead indicates acetylated BSA, higher molecular weight bands are acetylated BSA oligomers.

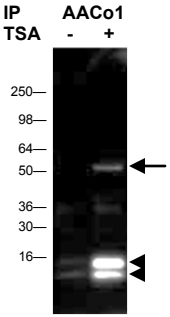
B) 20 mg of A431 cell lysate treated with TSA (+) or untreated (-). Strongly enhanced bands at 55 and 14-16 kD in TSA-treated lysate correspond to acetylated tubulin and histone proteins, respectively.

Immunolocalization: Lot 013



Human epidermal carcinoma A431 cells, untreated (left) or treated (right) with TSA (5 mM for 16 h), were permeabilized, fixed and probed with a 1:200 dilution of AAC01 as described in the material data sheet. Acetylated cytoplasmic and nuclear proteins were visualized in green fluorescence. Note that in contrast with the untreated control, acetylated microtubule network is clearly visible in TSA-treated sample. The fluorescent nuclear intensities indicate the high abundance of acetylated proteins in the nucleus.

Immunoprecipitation: Lot 013



1°: AAC01
2°: Clean Blot

A431 cells were either treated (+) or untreated (-) with TSA (0.6 µM for 6 h). Cell lysates were prepared in a modified RIPA buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% IGEPAL, 0.1% SDS, 0.5% Na deoxycholate) and 1.5 mg of lysate per reaction was used for IP of acetylated proteins. Each IP was performed as described in the material data sheet. Blots were developed using AAC01 at 1:500 dilution. Arrow points to acetylated tubulin. Arrowheads point to acetylated histones.