Certificate of Analysis

Product: **Anti-ubiquitin Mouse MAb** V 1.0

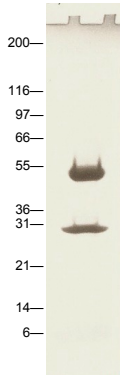
Catalog #: AUB01
Product Description: Mouse monoclonal IgG1
Clone P4D1
Purified by protein G affinity chromatography
Amount: 100 µl per tube when reconstituted
Lot: **001**

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.	71 µg
Sensitivity/Specificity	1 ng bovine thymus ubiquitin detected on western blot by chemiluminescence when 1:500 Ab dilution and 20 second exposure time is used.	1 ng detected
Endogenous specificity	Detects enhanced protein ubiquitination in Swiss 3T3 cells treated with 10 µM MG132 for 5h. Ubiquitin signal in 64-250 kD region is enhanced by ≥3 fold in western blot analysis of a 20 µg sample of Swiss 3T3 cell lysates. AUB01 used at 1:500 dilution, 20 second exposure for chemiluminescence detection.	≥3 fold enhancement of ubiquitin signal in 64-250 kD region.
Immunolocalization	Detects nuclear and cytoplasmic ubiquitin in HeLa cells. Nuclear signal is ≥3 fold higher than cytoplasmic signal.	Nuclear signal is ≥3 fold higher than cytoplasmic signal.
QC Release Date	12/12/14	

Kim Middleton
QC Manager

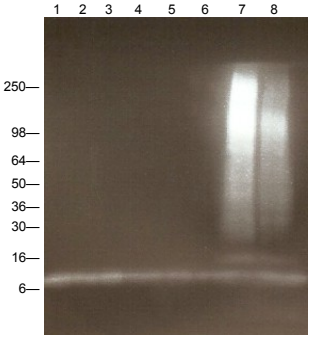
Lot specific QC Data

Purity Analysis: Lot 001



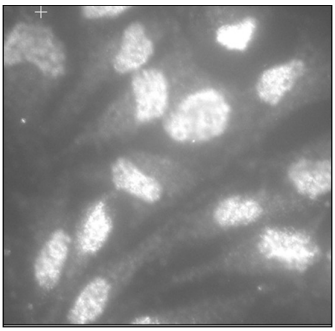
Purity analysis of protein G purified AUB01. 20 µg of AUB01 was run on 4-20% SDS-PAGE. Proteins were stained with coomassie blue. Bands at 22 kD and 55 kD represent antibody heavy and light chains respectively. The band at approximately 150 kD is non-reduced antibody.

Sensitivity/Specificity: Lot 001



Legend: AUB01 was used at a 1:500 dilution following the recommended Western blot protocol (see data sheet). Bovine thymus ubiquitin was run as follows; Lane 1-20 ng, Lane 2-10 ng. Lane 3–7.5 ng, Lane 4-5 ng, Lane 5-2.5 ng, Lane 6-1.0 ng Lanes 7 & 8 represent 20 ug of Swiss 3T3 cell lysate from cells treated for 5h with 10 µM MG132 (Lane 7) or untreated cells (Lane 8). Arrow indicates free ubiquitin band (8 kD), higher molecular weight bands are ubiquitinated proteins.

Immunolocalization: Lot 001



Legend: HeLa cells were stained and visualized by fluorescence microscopy following the recommended IF protocol (see data sheet). Ubiquitin targeted cytoplasmic and nuclear proteins and free ubiquitin were stained using AUB01 at 1:500 dilution.