Certificate of Analysis

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

Catalog #: APY03
Product Description: Mouse monoclonal IgG2b
Clone 27B10.4
Purified by protein G affinity chromatography
Amount: 100 µl per tube when reconstituted (APY03)
25 µl per tube when reconstituted (APY03-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	60-180 µg per tube Cat # APY03 15-45 µg per tube Cat# APY03-S Aliquot size is determined by biological activity therefore µg amounts per tube will vary between lots.	126 µg 32 µg
Sensitivity/Specificity	10 ng of chemically labeled phosphotyrosine-BSA detected on western blot by chemiluminescence when 1:500 Ab dilution and 30 second exposure time is used. A 1,000 ng sample of unlabeled BSA is not detected under identical conditions.	10 ng phosphotyrosine-BSA detected. 1,000 ng BSA not detected
Endogenous specificity	Detects multiple phosphoproteins in western blot analysis of 10 µg of cell lysate from NIH3T3 cells treated with pervanadate. Extremely faint or no signal is detected in untreated NIH3T3 or calyculin A treated A431 cell lysates. APY03 used at 1:500 dilution, 30 second exposure for chemiluminescence detection.	Multiple phosphoproteins detected in pervanadate treated NIH3T3 cell lysates. Faint or no signal in untreated or calyculin A treated cell lysates.
Immunolocalization	Pervanadate treated NIH3T3 cells show strong cytoplasmic staining, untreated cells show very faint cytoplasmic staining. APY03 used at 1:1000 dilution with 40 ms exposure time.	Enhanced phosphotyrosine-protein staining in pervanadate treated NIH3T3 cells.
Immunoprecipitation	APY03 (5 µl) bound to protein G-beads (30 µl) will specifically enrich phosphotyrosine-proteins from 200 µg of pervanadate treated NIH3T3 cell lysates.	Specific enrichment of phosphotyrosine proteins detected.
QC Release Date	2/19/15	

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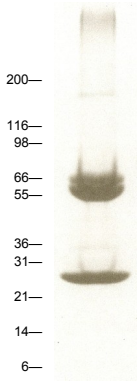
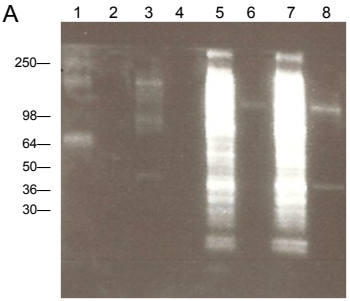
Lot specific QC Data

Purity Analysis: Lot 011



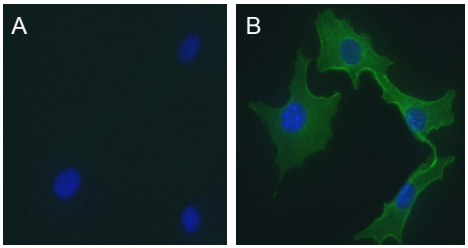
Purity analysis of protein G purified APY03. 20 µg of APY03 was run on 4-20% SDS-PAGE. Proteins were stained with coomassie blue. Bands at 22 kD and 55 kD represent antibody heavy and light chains respectively. NOTE: Antibodies are produced using ultra low IgG serum and there is negligible bovine IgG contamination. The heavy chain doublet is likely due to protein glycosylation which is a common occurrence in this isotype (Kim H. et al. 1994. J. Biol. Chem. 269: 12345 -12350).

Sensitivity/Specificity: Lot 011



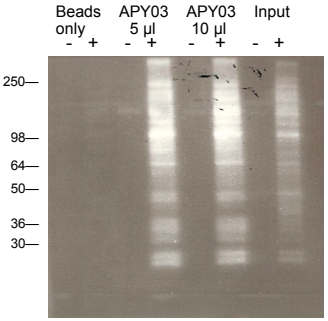
Lane 1: 10 ng phosphotyrosine labeled BSA, Lane 2: 1000 ng unlabeled BSA, Lane 3: 10 µg cell lysate from calyculin A treated A431 cells, Lane 4: 10 µg cell lysate from untreated A431 cells, Lanes 5 & 7: 10 µg and 20 µg respectively of cell lysate from pervanadate treated NIH3T3 cells (see data sheet for method), Lanes 6 & 8: 10 µg and 20 µg respectively of cell lysate from untreated NIH3T3 cells (see data sheet for method).

Immunolocalization: Lot 011



NIH3T3 cells, untreated (A) or treated (B) with sodium pervanadate (100 µM for 10 minutes), were permeabilized, fixed and probed with a 1:1000 dilution of APY03 as described in the material data sheet. Slides were developed using a 1:500 dilution of Alexa Fluor 555 labeled goat anti-mouse polyclonal secondary antibody. Nuclei were stained with 0.1 µg/ml DAPI.

Immunoprecipitation (IP): Lot 011



NIH3T3 cells were either treated (+) or untreated (-) with pervanadate (see data sheet for method). Cell lysates were prepared in RIPA buffer, each IP was performed using 200 µg of cell lysate and contained 30 µl of protein G beads plus the indicated amount of APY03 antibody. IPs were performed as described in the data sheet and western blots were developed using a 1:500 dilution of APY03 and a 1:1000 dilution of CleanBlot (Thermo Scientific) as secondary antibody. Input lysate represents 1/20th of IP lysate.