



Certificate of Analysis

Product: Anti-SUMO-2/3 Mouse Mab-agarose Beads V 1.1
Catalog #: ASM24-Beads
Product Description: Anti-SUMO-2/3 MAb crosslinked to protein G agarose beads
Clone 11G2
Amount: 400 µl per tube when reconstituted (ASM24-Beads)
Lot: 021

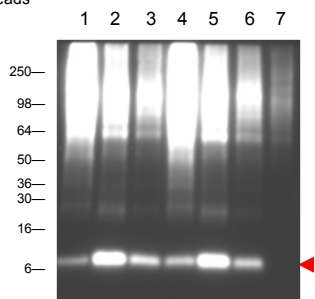
TEST	SPECIFICATON	LOT RESULTS
Appearance	Pink lyophilized powder	Pink lyophilized powder
Immunoprecipitation Sensitivity	Immunoprecipitation of SUMOylated Ubc9 protein from HeLa cell lysates. Signal strength from equivalent amounts of lysate; Heat shocked cells>untreated cells>SUMO-2 knockdown. Signals are comparable to previous Lot of ASM24-Beads.	SUMOylated Ubc9 protein detected. Heat shocked cells=untreated cells>SUMO-2 knockdown. Signals are comparable to previous Lot of ASM24-Beads.
Immunoprecipitation Specificity	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 ASM24-Beads.	Signal strength; heat shock>untreated>SUMO-2 knockdown. Signals comparable to previous Lot of ASM24.
Leaching of Ab light/heavy chains from beads	Crosslinking of Ab to beads has been optimized such that no leaching of light or heavy chains should be visible on western blot.	No Ab light or heavy chain visible in total SUMOylated protein or Ubc9 blot.
QC Release Date	7/13/15	

Kim Middleton
QC Manager

Lot specific QC Data

Immunoprecipitation & Detection of SUMOylated protein population: Lot 021

IP: ASM24-Beads
 WB: ASM23

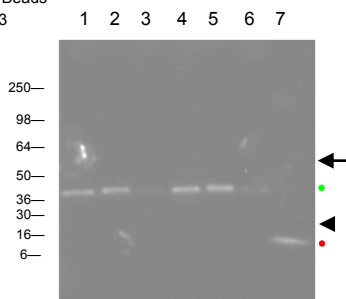


1 mg of cell lysate from heat shocked HeLa cells (Lanes 1 & 4), untreated HeLa cells (Lanes 2 & 5) and SUMO-2 shRNA knock-down HeLa cells (Lanes 3 & 6) were immunoprecipitated (IP) by incubation with 40 μ l of ASM24-Beads (anti-SUMO-2/3 antibody crosslinked to protein G-beads) according to the protocol in the product data sheet. Lanes 1-3 represent IP data using ASM24-Beads Lot 005, Lanes 4-6 represent IP data from ASM23-Beads Lot 021. Immunoprecipitated SUMO-2/3 modified proteins were run on SDS-PAGE, transferred to PVDF membranes and probed with a 1:500 dilution of ASM23. Exposure time 1 minute.

Red arrowhead indicates free SUMO-2/3. Lane 7 is 10 μ g of input lysate from heat shocked HeLa cells.

Immunoprecipitation & Detection of SUMOylated Ubc9 protein: Lot 021

IP: ASM24-Beads
 WB: ASM23



1 mg of cell lysate from heat shocked HeLa cells (Lanes 1 & 4), untreated HeLa cells (Lanes 2 & 5) and SUMO-2 shRNA knock-down HeLa cells (Lanes 3 & 6) were immunoprecipitated (IP) by incubation with 40 μ l of ASM24-Beads (anti-SUMO-2/3 antibody crosslinked to protein G-beads) according to the protocol in the product data sheet. Lanes 1-3 represent IP data using ASM24-Beads Lot 005, Lanes 4-6 represent IP data from ASM23-Beads Lot 021. Immunoprecipitated SUMO-2/3 modified proteins were run on SDS-PAGE, transferred to PVDF membranes and probed with a 1:500 dilution of Ubc9 antibody. Exposure time 1 minute.

Arrow indicates the position of Ab heavy chain, black arrowhead indicates the position of Ab light chain, it can be seen that no Ab heavy or light chains are visible in this western. Red arrowhead indicates free SUMO-2/3. Lane 7 is 10 μ g of input lysate from heat shocked HeLa cells. Red dot indicates molecular weight of non-modified Ubc9 (approx. 20 kD), green dot indicates molecular weight of SUMOylated Ubc9 (approx. 40 kD). This mobility shift agrees with previously published data¹.

References

1. Becker J. et al. 2013. Detecting endogenous SUMO targets in mammalian cells and tissues. *Nature Struct. & Mol. Biol.* 20, 525-531.