Improved immunoaffinity enrichment methods for arginine methylation

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INTRODUCTION

Immunoaffinity purification (IAP) coupled with LC-MS/MS is an established technique for the study of posttranslational modifications (PTMs). Recently we introduced an antibody-based magnetic bead conjugate IAP method that enables identification of sites of PTMs including lysine ubiquitination (KGG), lysine acetylation (AcK) and tyrosine phosphorylation (pY) with improved sensitivity and specificity over preexisting agarose bead methods. Here we present three novel magnetic bead IAP methods that enable identification of monomethylated (Me-R), symmetric dimethylated (SDMA), and asymmetric dimethylated (ADMA) arginine sites, with improvements over the respective agarose bead methods.

Arginine methylated proteins are involved in processes including transcriptional regulation and signal transduction. Arginine methylation is carried out by the arginine N-methyltransferase (PRMT) family of enzymes. Though all PRMT proteins catalyze monomethylation, Type I PRMTs such as PRMT1 add an additional methyl group to produce ADMA, while Type II PRMTs such as PRMT5 produce SDMA. Recent reports have highlighted the relevance of PRMT5, and SDMA, to cancer and stem cell self-renewal.

METHODS

Tryptic mouse and human peptides were used as input material for Me-R, SDMA, and ADMA magnetic IAP beads. Peptides were incubated with beads using cold 1x IAP buffer for 2 hours at 4°C. Beads were washed with cold 1x IAP buffer, then water, and bound peptides were eluted with trifluoroacetic acid. Eluted peptides were desalted using C18 Stage-Tips and analyzed on ThermoFisher Q-Exactive or Fusion Lumos mass spectrometers using data-dependent acquisition (DDA). Peptides were resolved using a 90 or 120min reversed phase gradient from 7.5 to 32% acetonitrile on a 25cm or 50cm C18 column. Peptides were identified by Comet with 1% of total identifications mapped to reverse sequences. Skyline software was used for MS1 peak review and quantification. For automation on Kingfisher Apex robot, input peptides were resuspended in bulk and aliquoted to deep well plates. After IAP on Kingfisher Apex robot, all enriched peptides were manually desalted as described above.

CONCLUSIONS

The new Me-R, SDMA, and ADMA magnetic IAP beads provide 6-60% improvement in number of unique modified peptides identified and nearly 100% improvement in PTM capture specificity across a variety of different cell and tissue sample types. The ease of handling of magnetic beads facilitates manual benchwork and implementation on automation platforms.

ACKNOWLEDGMENTS

We thank members of the CST Proteomics Department, Antibody Production, Molecular Biology, and Production Team 1 for their advice and discussions during the development of the PTMScan® HS antibodies.

PTMScan® HS workflow (Arginine methylated)

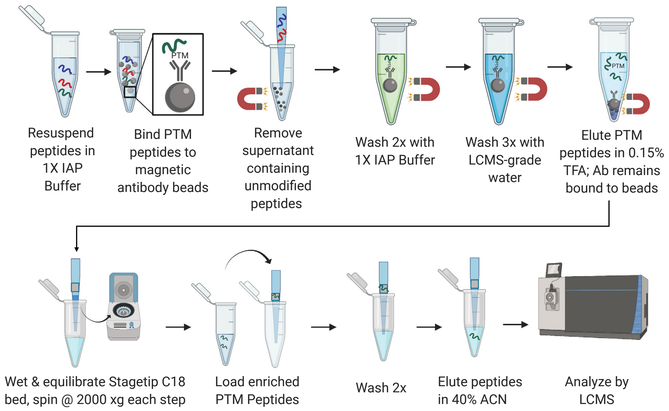

Figure 1: Users resuspend sample peptides prior to incubation with antibody magnetic beads. After washes, peptides are eluted and desalted prior to LCMS analysis.

Target Specificity of PTMScan® HS antibodies

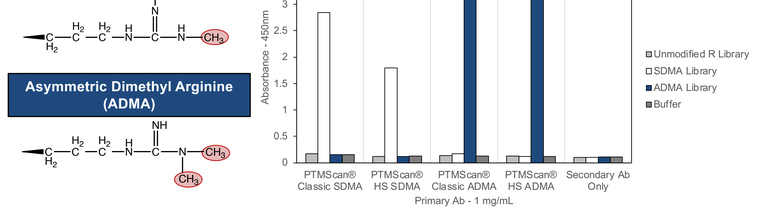

Figure 2: ELISA results with dimethyl-arginine PTMScan® antibodies that comprise agarose- ("Classic") and magnetic-bead formats ("HS"). Microwells were coated with degenerate peptide libraries or carbonate buffer as negative control. Data suggest PTM antibody specificity for recognizing the respective PTMs.

Pick Up Tip (96 DW tip comb); Tip Plate (DW 96)
Collect Beads, Count = 3, Collect Time = 1s; Beads plate (200uL, DW96)
Bind, Release beads = on, 4C & preheat = on, Mixing = 2hr slow; Peptides plate (1000uL, DW96).
Wait Forced plate change after this step = checked; Peptides plate (1000uL, DW96).
Wash 1, Release beads = on, 4C & preheat = on, Mixing = 20s slow, Collect beads = 3 count, 1s; Bound bead plate (1000uL, DW96).
Wash 2, Release beads = on, 4C & preheat = on, Mixing = 20s med, Collect beads = 3 count, 1s; IAP Wash 1 plate (1000uL, DW96).
Wash 3, Release beads = on, 4C & preheat = on, Mixing = 20s med, Collect beads = 3 count, 1s; IAP Wash 2 plate (1000uL, DW96).
Wash 4, Release beads = on, 4C & preheat = on, Mixing = 20s med, Collect beads = 3 count, 1s; Water 1 plate (1000uL, DW96).
Wash 5, Release beads = on, 4C & preheat = on, Mixing = 20s med, Collect beads = 3 count, 1s; Water 2 plate (1000uL, DW96).
Wash 6, Release beads = on, 4C & preheat = on, Mixing = 20s med, Collect beads = 3 count, 1s; Water 3 plate (1000uL, DW96).
Elute, Release beads = on, Mixing = 20sec slow, Collect beads = 3 count, 1 s; TFA plate (110uL, DW96).
Leave Tip; Water 3 plate (1000uL, DW96)

Improved PTM enrichment performance with PTMScan® HS

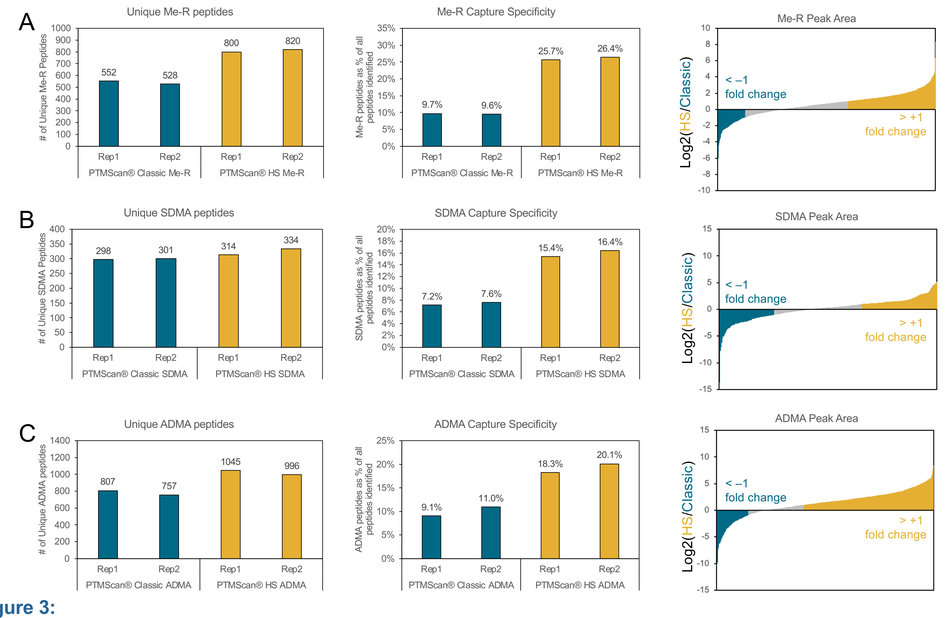

Figure 3:

A) Comparison of IAP performance between PTMScan® Classic and HS Me-R beads using 1mg of mouse liver input peptides, analyzed on Q-Exactive instrument. Label free quantification of peak areas of shared Me-R peptides detected at the MS1 level between HS and Classic pulldowns was performed to determine fold enrichment.

B) Comparison of IAP performance between PTMScan® Classic and HS SDMA beads using 1mg of human Hep G2 input peptides, analyzed on Q-Exactive instrument. Label free quantification of peak areas of shared SDMA peptides detected at the MS1 level between HS and Classic pulldowns was performed to determine fold enrichment.

C) Comparison of IAP performance between PTMScan® Classic and HS ADMA beads using 1mg of human HCT 116 input peptides, analyzed on Fusion Lumos instrument. Label free quantification of peak areas of shared ADMA peptides at the MS1 level between HS and Classic pulldowns was performed to determine fold enrichment.

Automation of PTMScan® HS

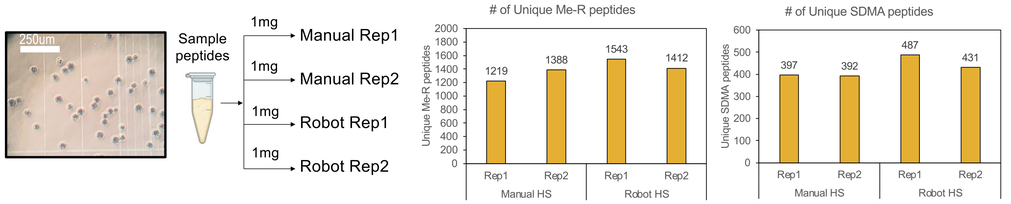

Figure 4: PTMScan® HS antibody-magnetic bead conjugates (microscopic image) are readily handled by automation platforms, including the ThermoFisher Kingfisher Apex robot. Automation reduces hands-on bench-time and promotes reproducible performance across experimenters. Shown are results from duplicate samples in separate wells prepared with PTMScan® HS Me-R and SDMA antibodies handled manually or using robot, suggesting comparable IAP performance. Summary of steps is provided to the left.

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