MagReSyn® Streptavidin MS: enabling more sensitive affinity workflows

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HIGHLIGHTS

- Protease-resistant MagReSyn® Streptavidin MS engineered for applications requiring on-bead digestion
- Multiple-fold reduction in streptavidin contaminating peptides during on-bead trypsinization
- Fewer contaminating peptides compact dynamic range thereby improving detection of target peptides
- Reduced non-specific binding of background proteins and peptides compared to standard product
- Retention of >80% of the capacity of standard MagReSyn® Streptavidin
- Workflow is fully automated on the Agilent Bravo liquid handling system

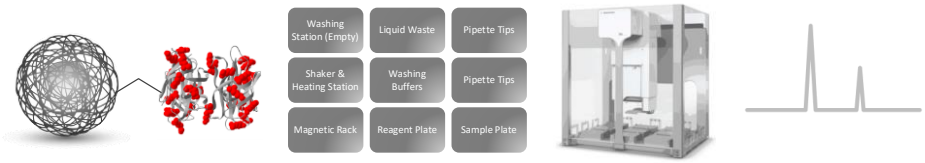
INTRODUCTION

To understand cellular function, it is critical to characterize interactions among biomolecules. One common strategy for proximity-based protein interaction analysis (APEX, BioID, LUX-MS etc) is based on the enrichment of biotinylated peptides/proteins using a streptavidin matrix, such as MagReSyn® Streptavidin. The very high affinity between biotin-tagged targets and streptavidin allows for the enrichment and subsequent detection of interaction partners while stringent washing conditions reduce non-specific binding. This strategy works well if the bound proteins/biomolecules can be eluted using harsh conditions, such as boiling the MagReSyn® Streptavidin beads in Laemmli buffer.

However, for mass-spectrometry-based (MS) identification, an on-bead digestion of bead-bound biotinylated proteins is typically preferred/employed. In such a digestion process, affinity-enriched proteins are digested, but also parts of the streptavidin matrix. In the subsequent LC-MS analysis, streptavidin-derived peptides mask the detection of low-abundance peptides of interest.

To circumvent these concerns, the new MagReSyn® Streptavidin MS is specifically designed to cater to applications requiring on-bead enzymatic digestion of proteins. This is achieved through the chemical modification of lysine and arginine residues of the immobilized Streptavidin as described by Rafiee *et al.* in 2020. (DOI: 10.15252/msb.20199370). The derivatization was optimized to retain a high affinity for biotin, retaining over 80% of the capacity of the standard MagReSyn® Streptavidin product, with multiple-fold decreases in the release of streptavidin peptides resulting from on-bead digestion.

METHODS



Evaluating binding capacity and reduction in streptavidin peptides from on-bead digest

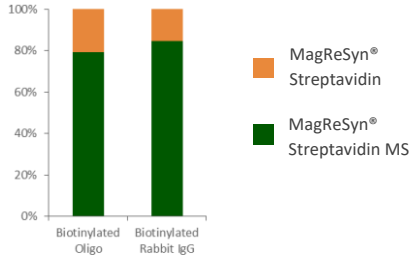
- The binding capacity of MagReSyn® Streptavidin MS was determined by incubating beads with pure biotinylated rabbit IgG and biotinylated oligo (15mer) to estimate capacity before and after chemical treatment of lysine and arginine residues.
- On-bead digestion was performed by incubating 20 µl of MagReSyn® Streptavidin or MagReSyn® Streptavidin MS, pre-equilibrated in 25 mM Ammonium Bicarbonate pH 8, with Trypsin (1:20) for 16 hours at 37°C.
- Digests were loaded on 75 µm x 2 cm Acclaim™ PepMap™ trap column using 2% ACN, 0.2% FA and separated via 15 min linear gradient on Waters CSH 75 µm x 25 cm column coupled to Sciex 6600 TTof operated in SWATH mode (48VW).
- Targeted data extraction for five streptavidin-derived peptides was performed in Skyline with sum of transition peak area utilized as a measure of peptide abundance.

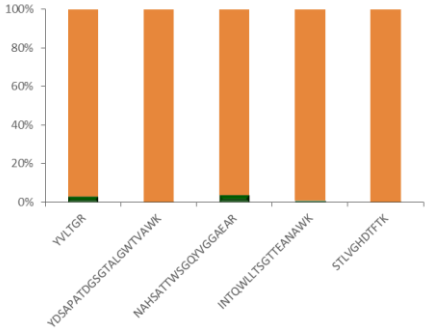
Binding capacity and reduction in streptavidin-derived peptides in a spiked cellular lysate

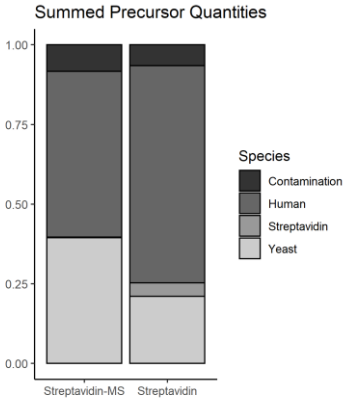
- The workflow was automated on an Agilent Bravo liquid handling station.
- Biotinylated target molecules were captured by incubating 15 µl MagReSyn® Streptavidin, or 16.5 µl MagReSyn® Streptavidin MS (corrected for capacity) with 400 µg of 1% NHS-biotinylated yeast protein spiked into HEK lysate for 20 minutes (quadruplicate samples).
- Non-specifically bound proteins were removed by 3 consecutive washes consisting of:
    - Wash 1: 1% SDS in PBS
    - Wash 2: 8M Urea in 50 mM Ammonium Bicarbonate (pH 8).
    - Wash 3: 5 M NaCl
    - Wash 4: 100 mM NaHCO3
    - Wash 5: 80% Isopropanol
    - Wash 6: 100 mM Ammonium Bicarbonate (pH 8).
- Target release was achieved via on-bead digestion performed overnight in 100 µl of 0.1% RapiGest™ in 100 mM Ammonium Bicarbonate digest buffer using 0.8 µg of Trypsin.
- Peptides were desalted using C18 cartridges, dried and resuspended in 3% ACN, 0.1% FA. Equal amounts were separated at 250 nl/min over a 70 min gradient of 5 to 41% ACN, 0.1% formic acid (FA), on a 75 µm x 15 cm, in-house packed C18-column connected to a nano-flow HPLC (ThermoFisher Scientific). Mass spectra were acquired on an Orbitrap Q Exactive™+ operated in DIA mode using 18 m/z windows and staggered collision energy (nce=25,27,30).
- Raw data files were processed using DIA-NN with an in-silico-generated spectral library. All reported data was filtered at 1% FDR and normalized intensities are shown.

RESULTS

Binding Capacity: MagReSyn® Streptavidin MS showed a marginal decrease in binding capacity of <15% in comparison to the standard Streptavidin (below left), but with a concomitant >95% reduction in abundance of streptavidin-derived peptides after on-bead digestion (below right).

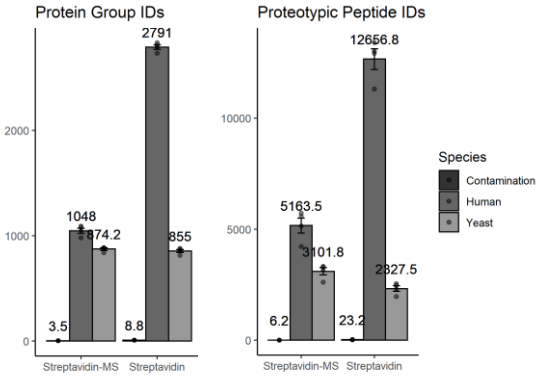


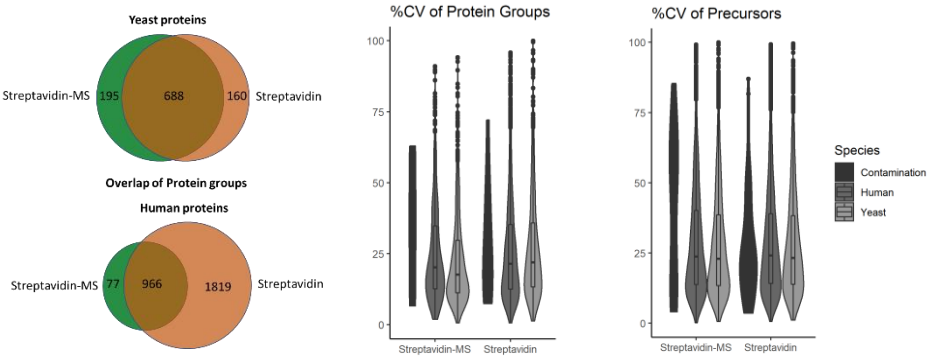




Isolation of biotinylated yeast proteins: Fewer streptavidin precursors, and with significantly lower intensity, were detected after the on-bead digestion with MagReSyn® Streptavidin MS after isolating biotinylated yeast proteins from a complex HEK cell lysate. This resulted in a higher number of yeast-derived target precursors, while the number of contaminating human background precursors decreased.

Samples enriched using MagReSyn® Streptavidin MS resulted in a similar number of yeast proteins being identified, but with more peptides isolated per protein, while the number of human-derived, contaminant peptides decreased significantly.





Over 80% of the target proteins detected in yeast overlapped between the standard Streptavidin and Streptavidin MS products (above left), but the technical reproducibility increased with median CV of 18% (Streptavidin MS) versus 22 % (standard Streptavidin – violin plots above).

CONCLUSIONS

- ✓ We describe a new digestion-resistant MagReSyn® Streptavidin MS product
- ✓ The binding capacity for biotinylated IgG shows only a marginal decrease, but with the vast reduction in the abundance of streptavidin-derived peptides after on-bead digestion
- ✓ When evaluated for the capture of NHS-biotinylated yeast proteins in a complex lysate, the result was an increased number of identifications and increased confidence in quantitation.
- ✓ This in turn resulted in an overall increase in the reproducibility of the workflow.
- ✓ The chemical modification of lysine and arginine residues in streptavidin, had a further positive effect by improving the selectivity over non-treated, which could possibly be attributed to reduction in ionic interactions with the lysate.

REFERENCES

R Studio – Available online: https://posit.co/download/rstudio-desktop/
Rafiee et al., 2020. Mol. Systems Biol. DOI: 10.15252/msb.20199370

Agilent Bravo scripts available on request.