Smart Notes

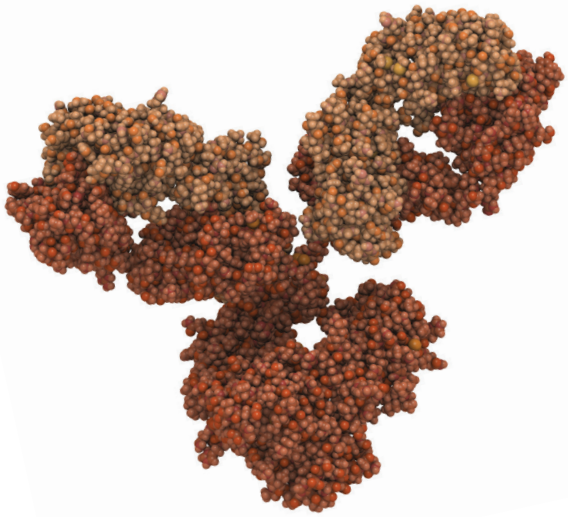
Increased reproducibility and performance of hybrid immunoassay workflows compared to bead based approaches.

Q&A

Have immunoassay workflows for the mass spectrometric (MS) bioanalysis of therapeutic monoclonal antibodies (mAb) evolved beyond conventional magnetic bead-based approaches?

Yes. The Thermo Scientific™ streptavidin MSIA™ D.A.R.T.'S provides a convenient and robust solution for the purification of therapeutic antibodies residing in complex biological samples, including mouse or human plasma, for their downstream analyses by mass spectrometry.

For effective purification of therapeutic antibodies from complex biological matrices, the methodology must provide low background, high recoveries, and be robust to enable reproducible results. The Thermo Scientific™ streptavidin MSIA™ D.A.R.T.'S are made of a porous monolithic column positioned at the distal end of a pipette tip and functionalized with covalently immobilized recombinant streptavidin. The chemistry utilized provides low non-specific binding of biological proteins, resulting in low background during LC-MS analyses. Furthermore, the devices are manufactured to reduce variation, allowing for high reproducibility within and between lots.



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How can therapeutic antibodies residing in complex biological matrices be more efficiently purified for hybrid workflows?

Limiting Background Impact

Background has a significant impact on the sensitivity and reproducibility of an assay. Therefore, it is important non-specific binding is minimal when purifying therapeutic antibodies from complex biological matrices. Background also contributes to dirtier samples being loaded onto your LC, increasing the workload on the LC for separation and reducing the lifetime of your LC columns.

As illustrated in Figure 1, in a recent study, MSIA D.A.R.T.'S provided on average 50 percent less background than the beads.

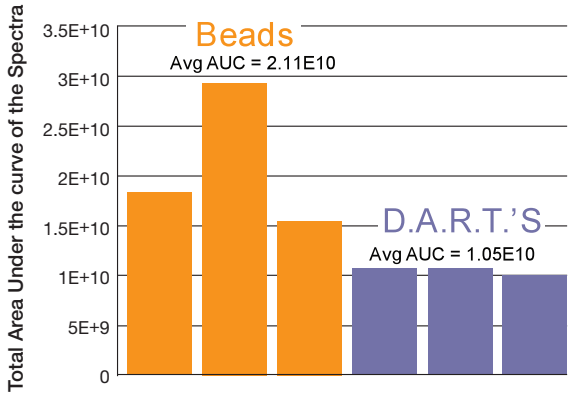

Figure 1. Background originating from plasma samples in the absence of the targeted therapeutic antibody.

Increased signal and improved reproducibility

Efficient recovery is required to improve assay dynamic range and sensitivity. The functional MSIA D.A.R.T.'S format improves binding kinetics by utilizing micro-fluidic properties to significantly increase the number of occurrences the therapeutic antibody comes in contact with the anti-Fc antibody immobilized to the streptavidin. Through repetitive pipetting of the sample volume, which can be automated using an automated liquid handler, the therapeutic antibody residing within the biological matrix can be forced to interact with the anti-Fc located on the streptavidin surface. This results in improved kinetics and thus increased extraction efficiency.

As shown in Figure 2, in a recent study, streptavidin MSIA D.A.R.T.'S provided on average 47 percent more of the therapeutic antibody than the streptavidin beads. In addition, the MSIA D.A.R.T.'S demonstrated to provide greater reproducibility with percent CVs of 7.5%, in comparison to the 37% achieved by the bead based method.

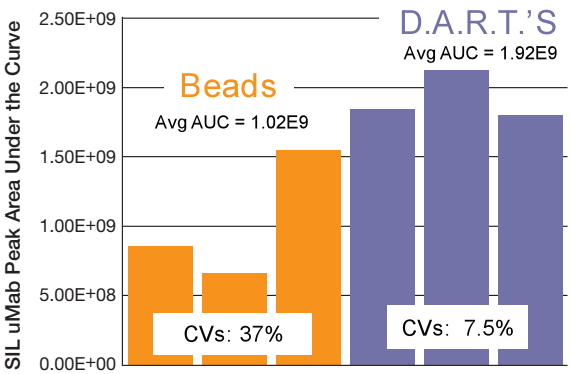

Figure 2. Signal generated from the extraction of 1 µg of therapeutic antibody from 250 µL of mouse plasma.

Summary

A 47 percent increase in signal of mAb, combined with a 50 percent decrease in background, resulted in the streptavidin MSIA™ D.A.R.T.'S providing more reproducible and superior performance to that of the bead based method.

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Contact Information **USA** +1 800 532 4752

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