Affimer Anti-idiotypic Binders: Critical Reagents For Clinical Ligand Binding Assays

POS025

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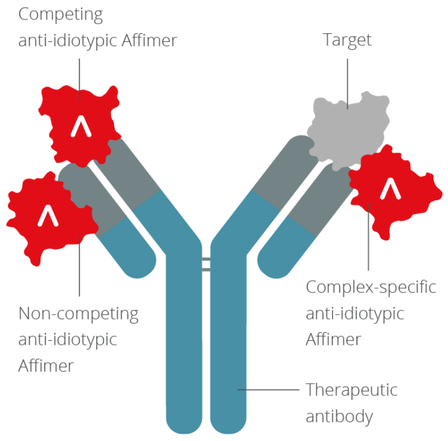
Critical Reagents for Ligand Binding Assays

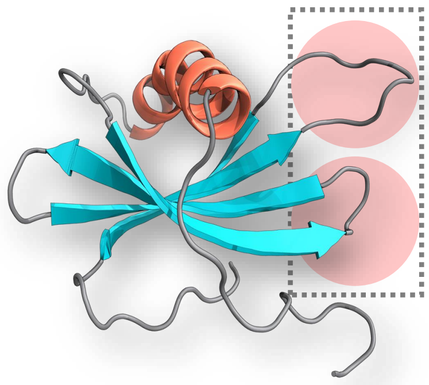
- This data demonstrates the next generation of anti-idiotypic reagents, based on the Affimer® scaffold, can significantly accelerate and improve the development of PK assays.
- Accurate quantification of therapeutic antibodies in biological matrices is essential for assessing pharmacokinetic properties.
- Fast and reliable supply of critical reagents is necessary to develop bioanalytical methods that can be used as part of FDA or EMEA approved guidelines.
- Various ligand binding assay formats have been explored to measure either free, bound or total antibody concentration depending on their therapeutic target.



Different types of anti-idiotypic reagents to measure bound, free or total antibody concentrations

The Affimer Scaffold

The Affimer scaffold is based on naturally occurring proteins (cystatins), engineered to stably display two loops creating a binding surface. Avacta has built large Affimer libraries (10^{10} – 10^{11}) which can be screened for binders to a broad range of targets using standard phage display techniques.



Affimers provide significant advantages for bioanalytical applications:

- **Speed of development**: high affinity reagents can be generated in three months without the need for affinity maturation
- **Reproducible supply**: based on robust and high yield recombinant bacterial production process
- **Low matrix effect**: exquisite specificity for three-dimensional conformations allows specific detection in complex background

Anti-Idiotypic Affimer Discovery and Validation

13 weeks

Phage Display

Trastuzumab and recombinant antibodies containing CDR-regions of Rituximab and Adalimumab were biotinylated and submitted to three rounds of phage display. An Affimer repertoire specific for each antibody was identified and 192 clones per targets were cloned and submitted to primary screen.

Primary Screen and Sequencing

All 192 clones were sequenced to identify unique clones and tested on a high-throughput bead assay for affinity and specificity.

ELISA Validation

A secondary screen was performed using direct capture ELISA of biotinylated target mAb with Strep-HRP detection.

Assay Performance Validation

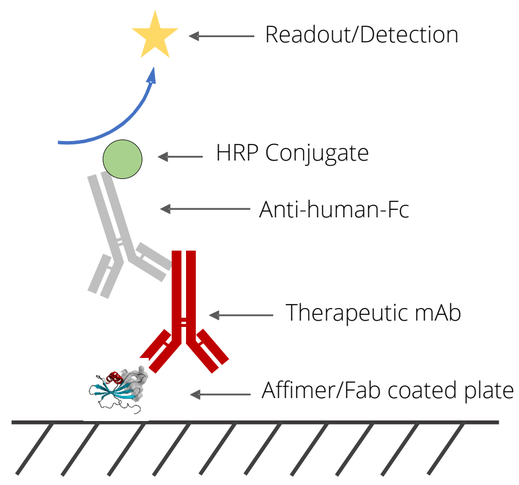
The best clones were validated in a sandwich ELISA using anti-isotype detection including:

- Specificity testing against other therapeutic antibodies
- Optimisation of dynamic range around clinical concentration
- Full titration in buffer and in 10% serum background to assess matrix effect
- Assay metrics including intra/inter assay variation, recovery, LLoQ, ULoQ and dynamic range

Performance of Affimer Proteins Versus Fabs

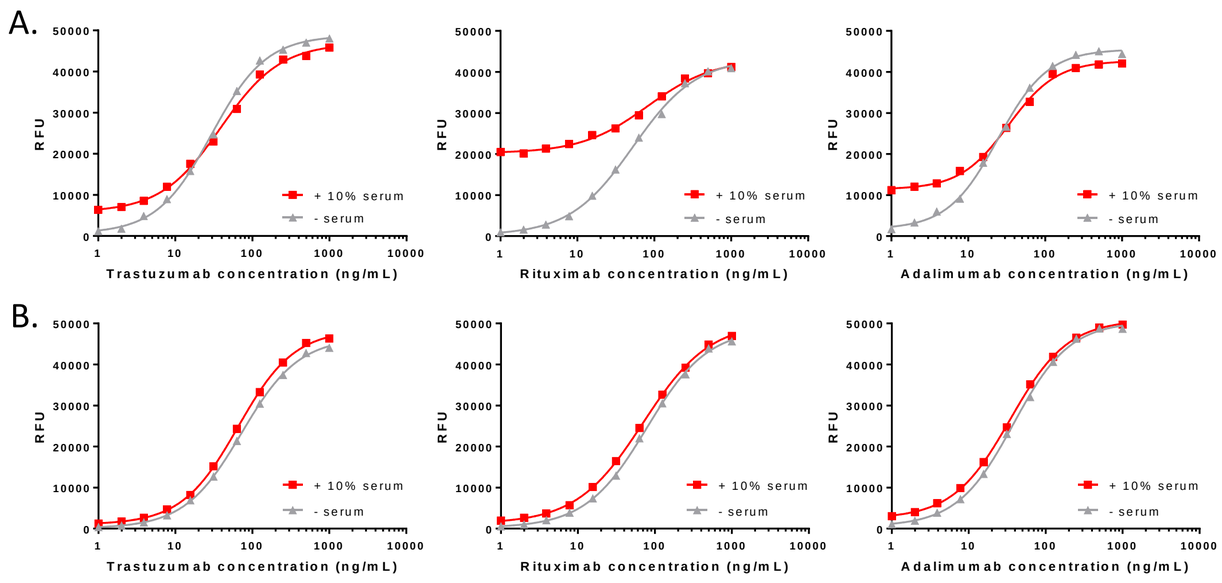
- Standard anti-idiotypic antibodies or fragments can lack specificity due to some level of cross reactivity to background hIgGs present at high concentration in serum.
- As a result, development of reliable assays often requires two specific anti-idiotypic reagents in a bridging format to attain performance meeting regulatory guidelines.
- Affimer reagents can be used in a simple, reliable and cost effective assay format.

Assay Format



Assay Conditions

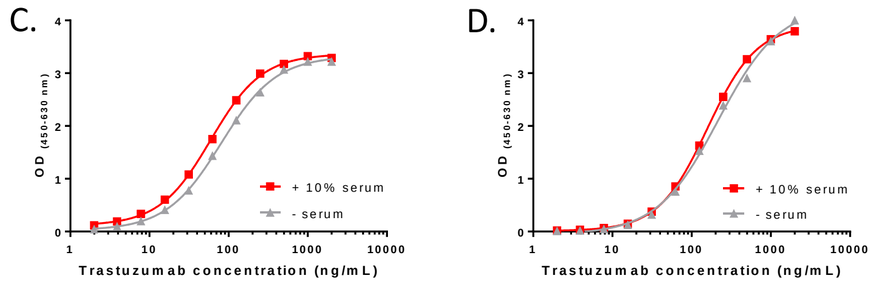
- Affimer or Fab passively absorbed on Maxisorp plate at a concentration of 1 µg/mL (Affimers) or 5 µg/ml (Fabs).
- Blocked with 1 x casein (Affimers) or 5% BSA (Fabs).
- Detected with HRP conjugated anti-Fc mAb.
- Visualised using QuantaBlu.



- In an assay using commercially available Fabs as capture reagents and an anti-isotype detection significant matrix effect is observed, leading to reduced dynamic range in the presence of serum (graphs A).
- When replacing the Fabs by anti-idiotypic Affimer binders, minimal matrix effect is observed (graphs B).

Flexible Anti-IgG Detection

- The commercially available HRP-conjugated anti-Fc detection antibody gives a dynamic range of 4-1000ng/mL with intra-assay CV ≤ 24.6%, Bias ≤ 9.5%.
- A biotinylated anti-IgG Affimer protein, pre-incubated with streptavidin-HRP, can also be used as a detection reagent with lower matrix effect and equivalent performance (graph D). Dynamic range = 4-1000ng/mL. Intra-assay CV ≤ 20%, Bias ≤ 19%.



Conclusion

- The Affimer platform can be used to reliably produce anti-idiotypic capture reagents with significantly lower matrix effect compared to commercially available antibody fragments.
- With short development timeline, reliable supply, low batch-to-batch variation and simple assay design, they offer significant advantages for the development of cost-effective PK ligand binding bioassays.

For further information please contact affimers@avacta.com or visit www.avacta.com