Affimer® Anti-Idiotypic Binders: High-performing Critical Reagents for Diverse Clinical Needs

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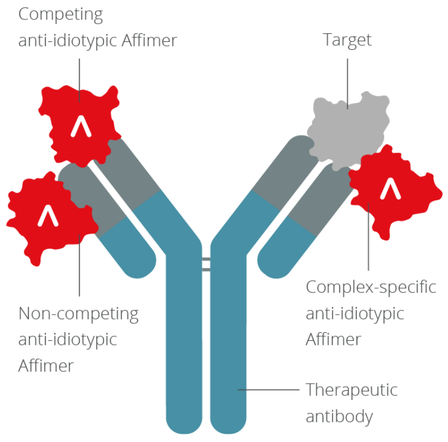
Critical Reagents for Ligand Binding Assays

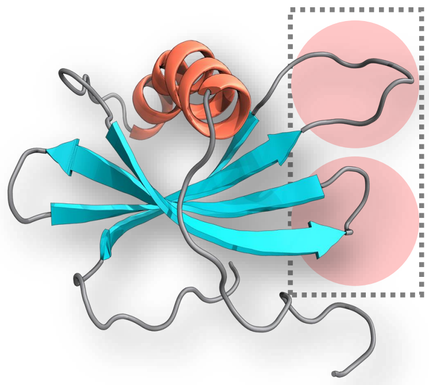
- Accurate quantification of therapeutic antibodies in biological matrices is essential for assessing pharmacokinetic properties.
- Fast and reliable supply of critical reagents is necessary to develop bioanalytical methods that can be used as part of FDA or EMA approved guidelines.
- Various ligand binding assay formats have been explored to measure either free, bound or total antibody concentration with Affimer reagents depending on their therapeutic target.
- 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.



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 13 weeks without the need for affinity maturation.
- **Reproducible supply**: based on a robust and high yield recombinant bacterial production process.
- **Low matrix effect**: exquisite specificity for three-dimensional conformations allows specific detection in complex backgrounds.

Anti-Idiotypic Affimer Discovery and Validation

13 Weeks

Phage Display

Recombinant antibodies containing CDR-regions of commercial therapeutic mAbs were biotinylated and submitted to three rounds of phage display. An Affimer repertoire specific for each antibody was identified and 192 hits per target were cloned and submitted to primary screen.

Primary Screen and Sequencing

For each target 192 hits were sequenced to identify unique clones. These were tested in 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 streptavidin-HRP detection.

Assay Performance Validation

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

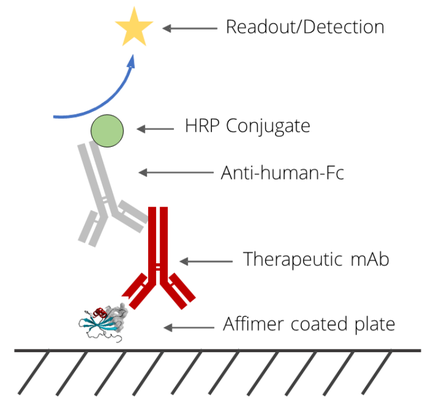
- Specificity testing against other therapeutic antibodies.
- Optimisation of dynamic range around clinical concentration.
- Full titration in buffer and serum matrix background to meet assay requirements.
- Curve metrics including intra-/inter-assay variation, recovery, LLoQ, ULoQ and dynamic range.
- Quality control (QC) samples spanning the range of the curve used to monitor assay performance.

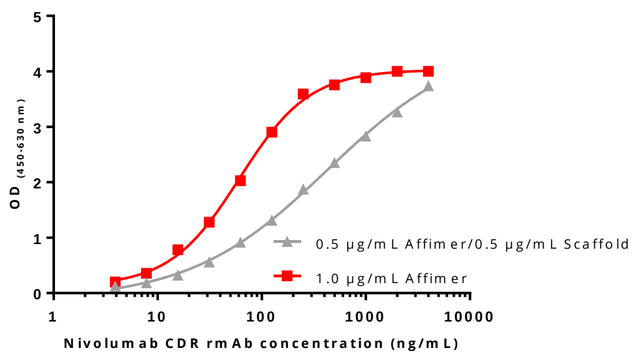
Tailoring Affimer Assay Performance

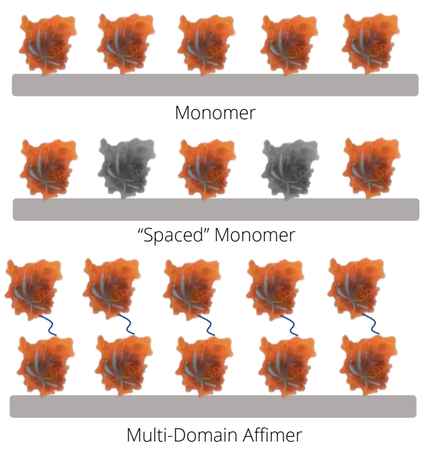
- The results of the screening process present a diverse repertoire of Affimer binders with varying affinities allowing selection of reagents to fit assay requirements.

Target	Clones Screened	Unique Sequences	Clones Selected
Rituximab	192	102	17
Adalimumab	192	15	15
Ipilimumab	192	113	16
Eculizumab	192	105	12
Nivolumab	192	26	10
Pembrolizumab	192	102	12

- Affimer reagents can be used in a simple, reliable and cost effective assay format that require only one specific anti-idiotypic reagent.
- Assay performance can be modulated using various surface options.
- All other assay conditions are fixed. This allows fine adjustments of dynamic range or a drive towards sensitivity.



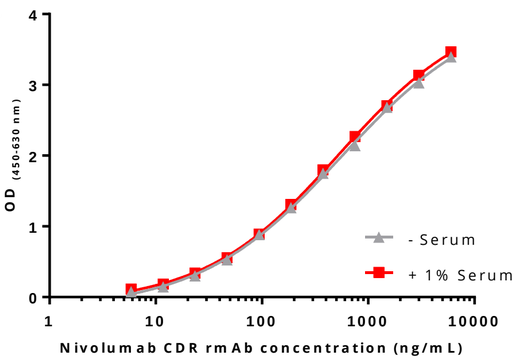




Nivolumab Affimer: Assay Performance

- Here we show PK performance of an Affimer that targets an anti-PD-1 mAb, Nivolumab, and report assay metrics (graph A), dilutional linearity (graph B) and specificity against other mAb therapeutics (graph C).

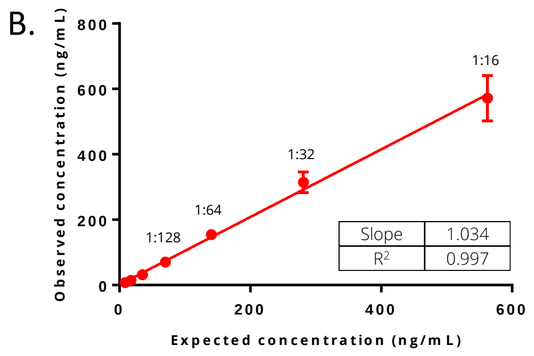
A.



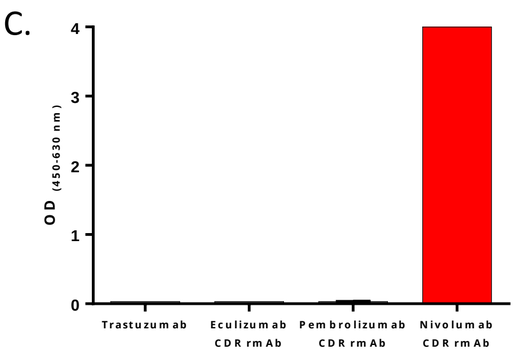
In 1% Serum	Nivolumab CDR mAb	
Calibration range (ng/mL)	ULOQ	3000
	LLOQ	5.9
Inter-assay calibration standard metrics	% CV	1.2 – 9.1
	% Recovery	89.2 – 125.6* (105.7)
Intra-assay calibration standard metrics	% CV	0.8 – 18.7
	% Recovery	86.0 – 123.5* (110.6)

* = Values approaching guideline limits are at LLoQ (LoQ values discounted)

B.



C.



Conclusion

- The Affimer platform can be used to reliably produce anti-idiotypic capture reagents with diverse binding affinities providing versatility for PK and drug monitoring assays. This allows flexibility to tailor assays to custom requirements.
- With short development timelines, reliable supply, minimal batch-to-batch variation and simple assay design, Affimer reagents offer significant advantages for the development of cost-effective PK ligand binding bioassays.