Turning a Negative to a Positive with Anti-metatype Affimer® Reagents.

POS035

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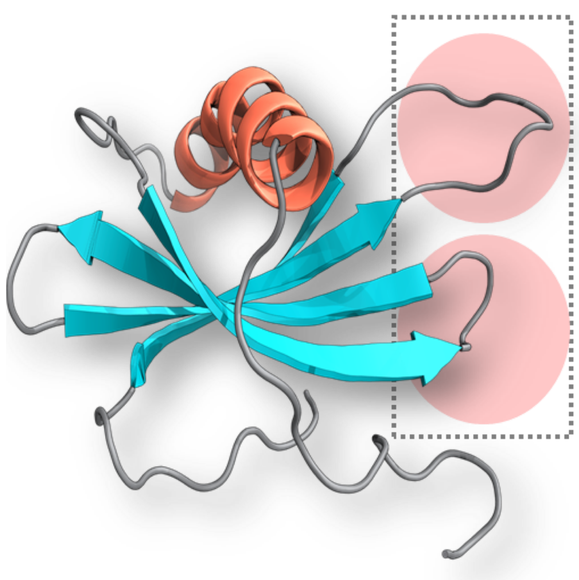
Critical Reagents for Ligand Binding Assays

The goal of our work has been to assess the capability of the Affimer platform to convert negative-read competition assays to positive-read sandwich assays for haptens. Currently available IVD immunoassays for haptens are almost all competition assays, thus associated with restricted sensitivity and specificity. Here we describe Affimer reagents that specifically recognise hapten-antibody complexes which can be used to convert competition assays into sandwich assays.

The Affimer Scaffold



- Based on naturally occurring proteins (cystatins).
- Currently operate with 2 scaffolds.
- Randomised binding loops (2x9aa) give rise to unique binding surfaces.
- Use phage display library (10^{10} – 10^{11}) to identify binders.
- Single domain proteins 1/10th size of antibody (14 KDa).
- Simple structure means production is rapid.
- Affimer reagents are easily engineered.

Affimer Reagent Discovery and Characterisation

Target Protein QC: Anti-estradiol antibody was biotinylated and biotinylation verified by SDS-PAGE & WB.

Phage Display: 3-rounds of phage display were performed against estradiol captured by immobilised, biotinylated, anti-estradiol antibody. Deselection was performed against biotinylated antibody alone to identify Affimer repertoires specific for the estradiol:antibody complex in preference to antibody alone.

Primary Screening: 192 clones were sequenced and screened in a high throughput bead assay and a capture ELISA for recognition of the complex and not the antibody alone.

ELISA Development: Lead clones were used to develop sandwich ELISA assays. This included matrix testing, specificity testing and calculation of intra-/inter-assay metrics to give LoQs and dynamic range.

LFD Development: A 2-domain Affimer reagent was conjugated to gold particles and the anti-estradiol antibody was plotted onto nitrocellulose. Wet assays were run with 20 µL buffer + 4 µL gold conjugate, dry assays were run with 60 µL sample (urine + estradiol). Results were read using an in-house reader which utilises a 0.5 megapixel camera and diffused light with line intensity read through the red frequency.

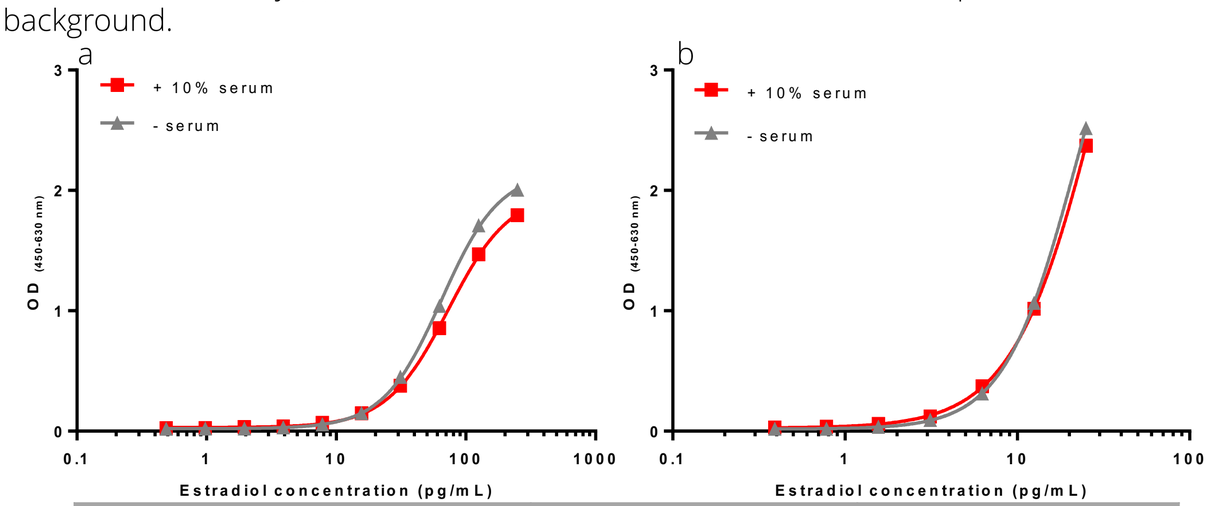
Primary Screen Data

The primary screen yielded 17 unique candidates that were specific for estradiol bound by an anti-estradiol antibody with no cross-reactivity with antibody alone.

	iQue								ELISA	
	Antibody alone				Antibody + Estradiol					
Affimer Working ID	Anti-Estradiol ab	Anti-Progest-	mIgG2b	No Target	Anti-Estradiol ab	Anti-Progest-	mIgG2b	No Target	Antibody Alone	Antibody + Estradiol
00468_666480	1689	1435	1523	1303.5	220807	1225.5	1699	1288.5	0.0169	2.0247
00468_666377	6815	1679.5	1722.5	1291	306013.5	1360.5	1685	1313	0.0174	2.5477
00468_666462	2067.5	1170	1420	989	127137	1450	2270	1455	0.0477	3.9398
00468_666430	1828	1357.5	1674	1434.5	188305	1325.5	1590	1423	0.0127	1.6804
00468_666382	2084	1639	1563	1219	117035	1710	1707.5	1337.5	0.0182	0.6475
00468_666496	2919	2537.5	1974	1130.5	77115.5	2194	1453	1299	0.0131	0.0311
00468_666465	1381.5	1362	1715	1192	208569	1488	2223	1257	0.0118	1.8166
00468_666395	2194	1561	2100	1742	108741	1442	1936	1773.5	0.0134	2.0452
00468_666482	1622	1348	1721	1125	147008	1395	1543	1289	0.0158	0.1916
00468_666386	1313	1098	1407	1132.5	1800	1293.5	1566	1018.5	0.0435	0.0405
00468_666427	4517	1445	1588	1301	236784	1475	1601.5	1351	0.0139	2.5892
00468_666365 ★	1976	1748.5	1627	1194.5	163628.5	1989	1909	1414.5	0.0647	3.9202
00468_666388	2226	1287.5	1689.5	1388	211869	1528	1903.5	1049	0.0173	3.9419
00468_666434	1268	1405	1768	1131	143080	1550	1909	1258.5	0.0196	0.0163
00468_666538	1391	1429	1779	1043	162456.5	1301.5	1723	1066.5	0.0108	0.2054
00468_666383	1654	1472	1762	1395.5	48072.5	1524	1580	1556	0.0201	0.0105
00468_666464	2617	1999.5	1982	1323.5	235359	1548	1762	1283	0.0156	3.94

ELISA Assay Performance and Matrix Effects

The selected candidate was used to develop 2 ELISA assays: a broad-range assay (a) and a sensitive assay (b). Both exhibit minimal matrix effects in a 10% pooled serum background.



		(a) Broad range Assay		(b) Sensitive Assay	
		- serum	+ 10% serum	- serum	+ 10% serum
Calibration range (pg/mL)	ULOQ	250	2500	12.5	125
	LLOQ	4	20	0.39	3.91
Inter-assay calibration standard metrics	% CV	0.6 – 2.2	1.0 – 9.8	0.2 – 4.5	0.6 – 18.8
	% Recovery	99.1 – 100.9	94 – 107.2	98.7 – 106.2	97.6 – 106.1
Intra-assay calibration standard metrics	% CV	2.7 – 16.8	0.1 – 16.7	1.9 - 13.5	1.0 – 24.7
	% Recovery	97.7 – 102.9	84.1 – 119.3	95.6 – 108.6	91.1 – 119.2

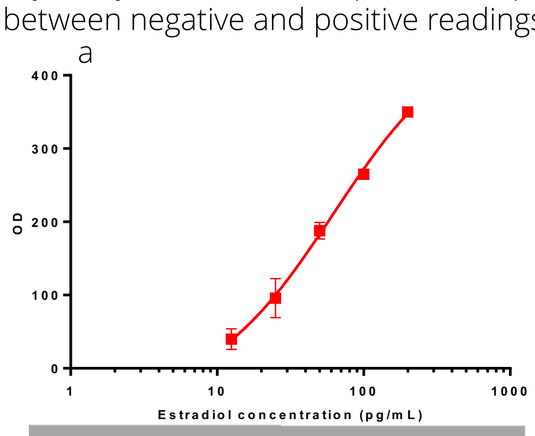
ELISA Assay Specificity

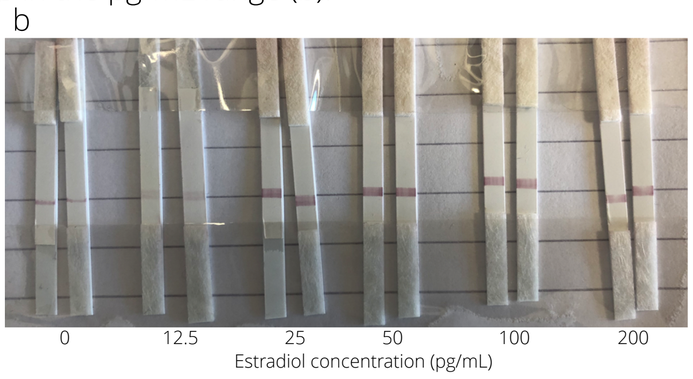
The ELISA assays are specific for the complex of estradiol and anti-estradiol, exhibiting minimal signal when other molecules were incubated with the anti-estradiol antibody. All molecules tested at 500 pg/mL.

Estradiol /α-Estradiol	Estrone /α-Estradiol	Estriol /α-Estradiol	Estradiol-3-glucuronide /α-Estradiol	Progesterone /α-Estradiol
100.0	2.7	0.4	0.0	2.2

LFD Assay Performance

Affimer reagents have also been assessed for use in a positive-read lateral flow sandwich assay. Development started with a quantitative wet assay which uses 40 nm gold particles and has an accurate and precise range of 12.5 to 200 pg/mL (a). A dry assay is also in development and preliminary data shows a distinct difference between negative and positive readings in the pg/mL range (b).





Curve Metrics		
Calibration range (pg/mL)	ULOQ	200 pg/mL
	LLOQ	12.5 pg/mL
Intra-assay calibration standard metrics	% CV	8.2 – 22.7*
	% Recovery	94.5 – 104.3

Conclusion

- Using the Affimer platform, we have successfully developed Affimer reagents specific to an estradiol-antibody complex, allowing the conversion of a competition assay into a positive-read assay.
- We have evaluated Affimer reagent performance in both ELISA and LFD assay formats, demonstrating the versatility and robustness of this novel platform.
- Our method offers the potential to quickly and simply identify reagents to improve routine assays for a broad range of haptens including drugs, hormones and vitamins.