E3*scan* Ligand Binding Assay Platform for Targeted Protein Degradation and PROTAC Discovery

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Abstract

E3 ligases have emerged as pivotal targets for drug discovery using the promising new paradigm of targeted protein degradation. This new paradigm includes both ligand binding-directed "reprogramming" of E3 substrate specificity approaches and a more directed approach, using small molecule proteolysis-targeting chimeras (PROTAC®), to selectively degrade disease-driving proteins. As there are hundreds of diverse putative E3 ligases with differentiated tissue expression, this new paradigm may well define a next dimension of precision medicine defined by an axis of tissue-specific activity. While there have been some early successes, the E3 drug discovery field has a significant unmet need for a standardized biochemical ligand binding assay platform. A platform is required that: 1. Can measure ligand binding across the E3 family using a standardized method enabling "apples to apples" comparisons; 2. Is highly scalable and rapid; 3. Has an exquisite dynamic range for the measurement of accurate K_D values as low as single digit picomolar (pM). Eurofins Discovery herein presents its novel E3*scan*™ technology that addresses each of these unmet needs. E3*scan*, based upon well-established KINOME*scan*® technology, has been successfully applied to diverse E3 ligases, including CRBN, VHL, MDM2, cIAP1, cIAP2, and XIAP, with many other E3 assays in progress. We shall present assay validation data for these targets, including data for ligands with K_D values in the low to mid pM range. In conclusion, we present Eurofins Discovery's novel E3*scan* platform that can enable accelerated screening and SAR analysis in the E3 drug discovery field, with rapid turnaround times for discovery library screens (20 business day TAT) and weekly SAR (5 business day TAT) and the largest assay panel available on a single technology platform. (PROTAC is a registered trademark of Arvinas)

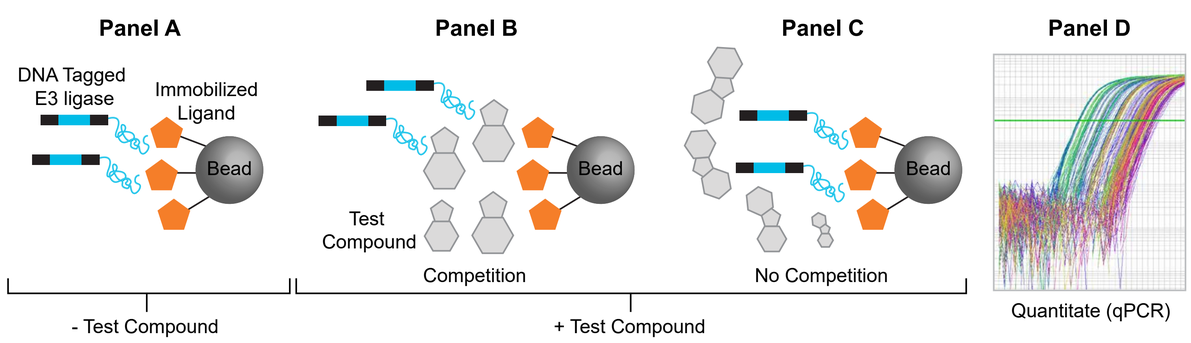
E3*scan* Assay Principle Using KINOME*scan* Platform

- There is a large unmet need for broad screening across the E3 ligase family using a single generic technology platform
- KINOME*scan* technology has been successfully applied to the development of generic substrate-recruitment site-directed competition binding assays for diverse E3 ligases

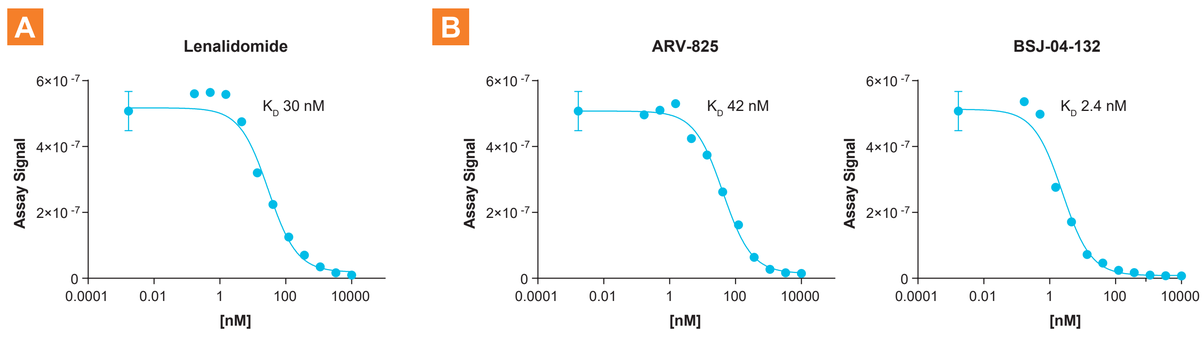
Three key components in the assay:

- E3 ligase tagged with DNA (low pM E3 concentration in assay)
  - Expression in mammalian cells or by using proprietary T7 phage display system
- Known E3 ligase ligand (small molecule or peptide) immobilized on solid support
- Test compound or solvent control

Measure amount of E3 ligase captured by solid support in the presence or absence of a test compound (ultrasensitive qPCR readout)



CRBN E3*scan* Assay



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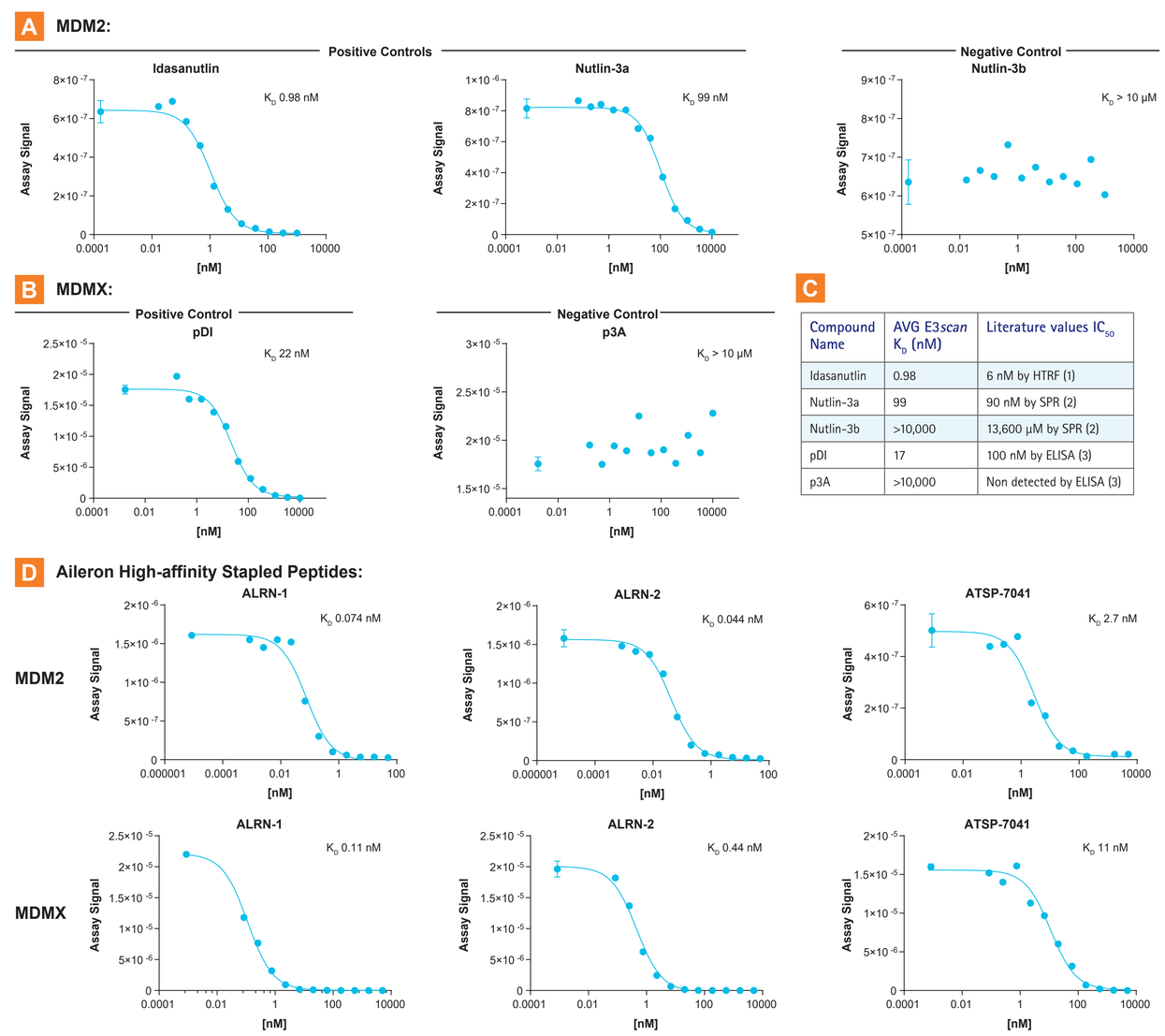
Compound Name	CRBN K_D (nM)	CRBN + DDB1 K_D (nM)
ARV-825	47	30
BSJ-03-123	1	5.6
BSJ-03-204	3.3	2.5
BSJ-04-132	2	1
dBET1	9.8	6.8
dBRD9	5.8	3.1
dTAG-13	140	79

Compound Name	CRBN K_D (nM)	CRBN + DDB1 K_D (nM)
Lenalidomide	28	13
Pomalidomide	45	26
THAL SNS 032	19	7.6
TL 12-186	13	5.2
TL 13-112	24	9.5
ZXH 3-26	2.4	1.6

Figure 1. K_D measurements from CRBN E3*scan* assay. Representative dose response curves for CRBN alone against A. control compounds or B. CRBN-targeting PROTACs. The amount of E3 ligase measured by qPCR (Assay Signal) is plotted against the corresponding compound concentration in nM. C. Data table summarizes K_D measurements of CRBN alone, or CRBN co-expressed with DDB1, against control compounds (Lenalidomide and Pomalidomide) and commercially available CRBN-targeting PROTACs.

MDM2 vs. MDMX E3*scan* assay



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Compound Name	AVG E3*scan* K_D (nM)	Literature values IC_{50}
Idasanutlin	0.98	6 nM by HTRF (1)
Nutlin-3a	99	90 nM by SPR (2)
Nutlin-3b	>10,000	13,600 µM by SPR (2)
pDI	17	100 nM by ELISA (3)
p3A	>10,000	Non detected by ELISA (3)

Figure 2. MDM2 vs. MDMX E3*scan* assay. K_D measurements for the interactions of (A.) MDM2 with positive control compounds, Idasanutlin, and Nutlin-3a, and negative control compound Nutlin-3b. and (B.) MDMX with positive control peptide pDI and negative control peptide p3A. C. Data table shows that the K_D values measured in this assay are comparable to reported literature values measured by other techniques (1-3). D. K_D measurements for the interactions of Aileron's stapled peptides with MDM2 and MDMX. The measured K_Ds for ATSP-7041 stapled peptide is comparable to literature values measured using Biacore, 0.91 and 2.31 nM, respectively (4).

VHL E3*scan* Assay

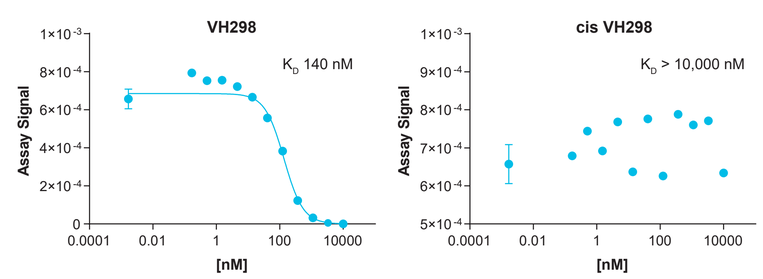

Figure 3. VHL E3*scan* assay. Curves represent K_D measurements for the interactions of positive control VH298 and negative control cis VH298 with VHL-elonginBC. VHL298 K_D values were similar plus/minus elonginBC. However, elonginBC greatly improved Assay Signal.

Summary

- We have developed and validated E3scan assays against MDM2, MDMX, VHL, CRBN, cIAP1, cIAP2, and XIAP
- All assays are robust & high throughput and give high quality K_D curves. Assay windows (signal/background) of ≥ 50-fold
- Correct potency and rank order for the control inhibitors tested
- Assays do not approach the tight binding limit – even for pM compounds and stapled peptides
- Assays can support an SAR campaign start to finish with minimal if any condition changes required for highly potent compounds

References

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