BROMO*scan*™ Profiling Service

Kd Report

Compound Name	Kds Determined
MN657	4
SIM1	4

Requester: Alessio CIULLI

Company: University of Dundee

Study Date: 04/09/2021

Report Date: 4/19/2021

Quote ID: US073-0016625-Q

Order ID: YIO001-01-s-00001

Product: bromoKdELECT

Number of Kds Determined: 8

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Technology Overview

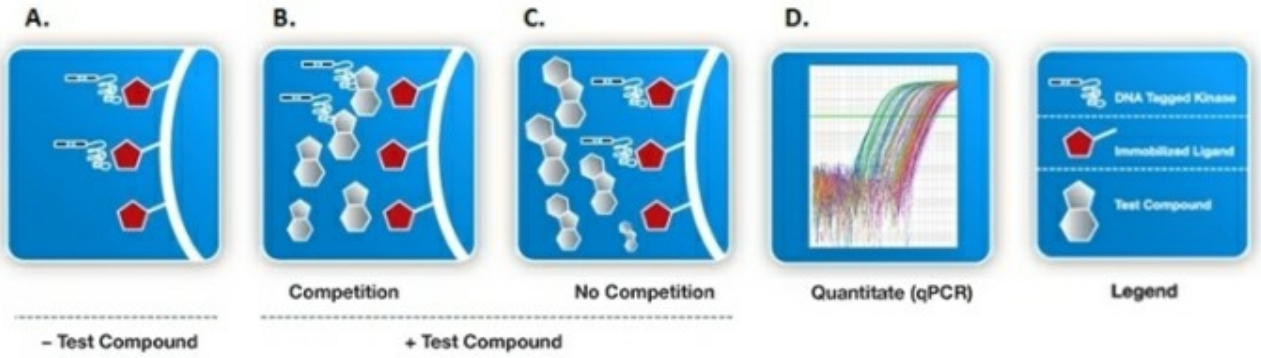
BROMO*scan*™ is a novel industry leading platform for identifying small molecule bromodomain inhibitors. Based on proven KINOME*scan*™ technology, BROMO*scan*™ employs a proprietary ligand binding site-directed competition assay to quantitatively measure interactions between test compounds and bromodomains. This robust and reliable assay panel is suitable for high throughput screening and delivers quantitative ligand binding data to facilitate the identification and optimization of potent and selective small molecule bromodomain inhibitors. BROMO*scan*™ assays include trace bromodomain concentrations (<0.1 nM) and thereby report true thermodynamic inhibitor Kd values over a broad range of affinities (<0.1 nM to >10 uM).

Assay Process

1. Assemble Assay Components:
   - *E. coli* or mammalian cell-expressed bromodomain labeled with DNA tag for qPCR readout
   - Known bromodomain ligand immobilized on a solid support
   - Test compound or control (solvent only)
2. Equilibrate
3. Wash solid support to remove unbound bromodomain
4. Quantify bromodomain captured on solid support (qPCR)
5. Compare captured bromodomain levels in test compound and control samples

Assay Principle

Compounds that bind the bromodomain prevent its binding to the immobilized ligand thus reducing the amount of protein captured on the solid support (A & B). Conversely, test molecules that do not bind the bromodomain have no effect on the amount of protein captured on the solid support (C). Screening "hits" are identified by measuring the amount of bromodomain captured in test versus control samples by using a quantitative, precise and ultra-sensitive qPCR method that detects the associated DNA label (D). In a similar manner, dissociation constants (Kds) for test compound-bromodomain interactions are calculated by measuring the amount of bromodomain protein captured on the solid support as a function of the test compound concentration.



Protocol Description

Bromodomain assays. T7 phage strains displaying bromodomains were grown in parallel in 24-well blocks in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (5,000 x g) and filtered (0.2µm) to remove cell debris. Streptavidin-coated magnetic beads were treated with biotinylated small molecule or acetylated peptide ligands for 30 minutes at room temperature to generate affinity resins for bromodomain assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining bromodomains, liganded affinity beads, and test compounds in 1x binding buffer (17% SeaBlock, 0.33x PBS, 0.04% Tween 20, 0.02% BSA, 0.004% Sodium azide, 7.4 mM DTT). Test compounds were prepared as 1000X stocks in 100% DMSO. Kds were determined using an 11-point 3-fold compound dilution series with one DMSO control point. All compounds for Kd measurements are distributed by acoustic transfer (non-contact dispensing) in 100% DMSO. The compounds were then diluted directly into the assays such that the final concentration of DMSO was 0.09%. All reactions performed in polypropylene 384-well plates. Each was a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 2 µM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The bromodomain concentration in the eluates was measured by qPCR.

Compound Handling

An 11-point 3-fold serial dilution of each test compound was prepared in 100% DMSO at 1000x final test concentration. All compounds for Kd measurements are distributed by acoustic transfer (non-contact dispensing) in 100% DMSO. The compounds were then diluted directly into the assays such that the final concentration of DMSO was 0.09%. Most Kds were determined using a compound top concentration = 10,000 nM. If the initial Kd determined was < 0.169 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration.

Binding Constants (Kds)

Binding constants (Kds) were calculated with a standard dose-response curve using the Hill equation:

$$\text{Response} = \text{Background} + \frac{\text{Signal - Background}}{1 + (\text{Kd}^{\text{Hill Slope}} / \text{Dose}^{\text{Hill Slope}})}$$

The Hill Slope was set to -1.

Curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm.

References

KINOME*scan*™ and BROMO*scan*™ use the same assay technology. For a more detailed description of this assay technology, see:

- Fabian, M.A. *et al*. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat. Biotechnol.* **23**, 329-336 (2005).

To view kinase interactions for 38 well-known kinase inhibitors, see:

- Karaman, M.W. *et al*. A quantitative analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **26**, 127-132 (2008).

For examples on how KINOME*scan* can opportunistically identify unanticipated therapeutically-beneficial interactions, see:

- Carter, T.A. *et al*. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc. Natl. Acad. Sci.* USA. **102**, 11011-11016 (2005)

For more information on the Hill equation and the Levenberg-Marquardt algorithm, see:

- Hill, A. V. The possible effects of the aggregation of the molecules of hemoglobin on its dissociation curves. *J. Physiol. (Lond.)*. **40**, iv-vii (1910).
- Levenberg, K. A method for the solution of certain non-linear problems in least squares. *Q. Appl. Math.* **2**, 164-168 (1944).

Select publications are available at www.discoverx.com.

YIO001-01-s-00001 Study Results

Table 1 - Matrix of Kds for YIO001-01-s-00001.

Target	MN657	SIM1
Gene Symbol	Kd (nM)	Kd (nM)
BRD2(1,2)	1.9	0.035
BRD3(1,2)	1.8	0.03
BRD4(1,2)	2.2	0.043
BRD4(full-length,short-iso.)	7.5	0.083

Kd Legend

x<100nM	100nM≤x<1uM	x≥1uM	No Binding	Not Requested

YIO001-01-s-00001 Curve Images

Table 2 - Curve Images for YIO001-01-s-00001. The amount of bromodomain measured by qPCR (Signal; y-axis) is plotted against the corresponding compound concentration in nM in log10 scale (x-axis). Data points marked with an "x" were not used for Kd determination.