

Bromodomain Assay Platform for Drug Screening and Discovery

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ABSTRACT: Epigenetic regulation has been studied extensively in the past few years, and many studies indicate that it is significantly involved in tumor establishment and progression. Epigenetic regulators are often referred to as writers, which covalently modify chromatin (e.g. methyltransferases or acetyltransferases), readers, which bind to modified sites (e.g. bromodomains bind acetyl-lysine residues), and erasers, which remove modifications (e.g. lysine demethylases or deacetylases). The BET proteins are targeted to acetylated histones by their two, tandem bromodomains and thereby affect chromatin structure and function. The binding of BET bromodomains to chromatin and BET protein recruitment of factors such as PTEFb can serve to regulate gene expression. After the discovery of JQ-1, a bromodomain inhibitor, and the validation of bromodomains as therapeutic targets, many efforts toward the development of new bromodomain-targeted drugs have begun. We have developed and successfully commercialized BET bromodomain binding assays using acetyl-lysine peptides as ligands and AlphaScreen technology for detection of binding. We will show assay development and compound profiling data obtained with several construct derived from the BET protein BRD4 (Bromodomain-containing protein 4). These include the isolated bromodomains 1 and 2, as well as a tandem bromodomain 1/2 and the full-length BRD4.

Bromodomain Assay was based on the Alpha Technology platform using His-tag BRD proteins and biotinylated acetylated peptides (Fig. 1). The His-tagged BRD4 proteins in different lengths (Fig. 2) were expressed in *E. coli*. For the titration studies, the 2-fold serial dilution of each BRD4 protein was incubated with the tetra-acetylated Histone H4 peptide (H4 (1-21) K5/8/12/16(Ac)4-Biotin) for 30 min in the reaction buffer containing 50 mM HEPES (pH 7.5), 100 mM NaCl, 0.05% CHAPS, and 0.1% BSA. The streptavidin-coated Donor beads, and then Ni-Chelate acceptor beads were added and incubated for 1 hour with gentle mixing. The signals were measured by EnVision Alpha detection. The IC_{50} determination of inhibitors was performed as above, but compounds were added by using acoustic technology (Echo550) into the BRD4 protein solution followed by 30 min preincubation. Further 30 min incubation after the addition of the tetra-acetylated peptide, and beads were added and the signal detection as above.

Fig. 1 AlphaScreen Bromodomain Assay

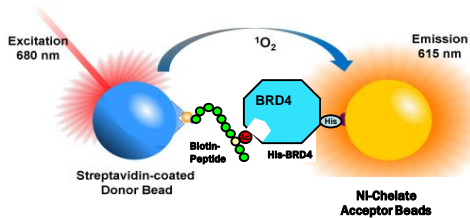
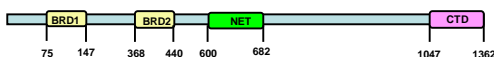


Fig. 2 BRD4 Structure



N-Terminus His-Tagged BRD4 Constructs:

- BRD4-1: aa 44-170
- BRD4-2: aa 349-460
- BRD4 Tandem: aa 44-460
- BRD4-FL: aa 1-1362

Fig. 3 Titration of BRD4 proteins against peptide concentration or protein concentration.

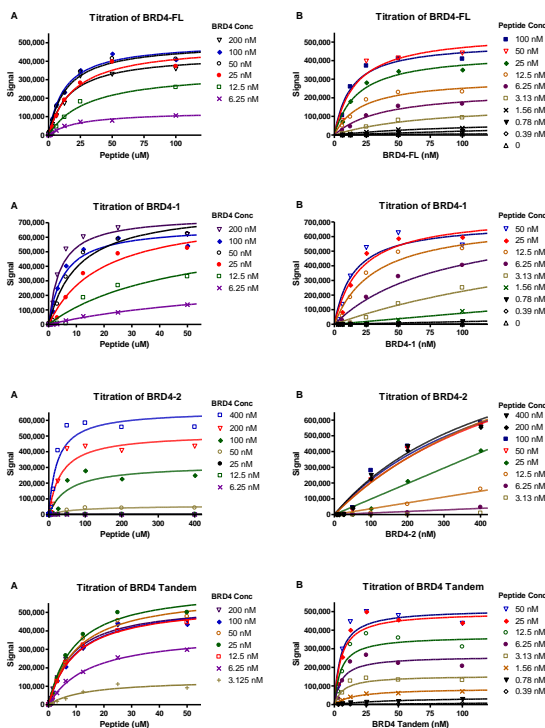


Table 1: Bromodomain Assay Conditions:

The conditions were defined from the titration data.

BRD	BRD Concentration (nM)	Peptide concentration (nM)
BRD4-FL	25	50
BRD4-1	25	25
BRD4-2	200	50
BRD4 Tandem	12.5	25

Fig. 4 DMSO Effect on AlphaScreen Bromodomain Assay.

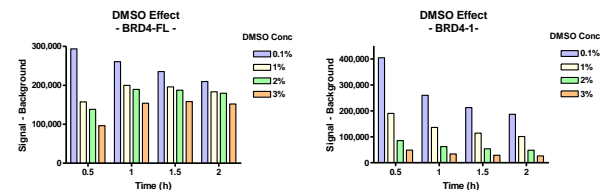


Fig. 5 DMSO Effect on IC50 values.

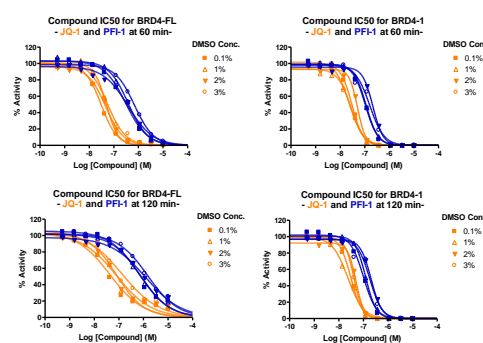


Table 2: JQ-1 IC50 values

DMSO	BRD4-FL		BRD4-1		BRD4-2		BRD4 Tandem	
	60 min	120 min	60 min	120 min	60 min	120 min	60 min	120 min
0.1%	28.44	57.18	32.13	31.32	66.77	65.59	929.0	1411
1%	42.47	92.20	27.63	29.37	28.26	31.02	1024	2050
2%	46.17	86.93	44.09	42.57	16.97	16.48	841.6	2126
3%	58.20	116.6	32.42	31.97	18.11	18.40	1186	2408

Table 3: PFI-1 IC50 values

DMSO	BRD4-FL		BRD4-1		BRD4-2		BRD4 Tandem	
	60 min	120 min	60 min	120 min	60 min	120 min	60 min	120 min
0.1%	338.6	748.6	130.4	159.6	434.7	431.8	796	7652
1%	389.2	729.2	159.9	112.9	181.5	223.5	8179	9227
2%	421.7	1125	184.0	190.0	267.7	257.0	7500	9921
3%	610.5	1449	206.9	198.9	220.8	227.8	9359	4149

CONCLUSIONS:

- AlphaScreen bromodomain assay is sensitive to DMSO concentration.
- DMSO sensitivity is different in different construct, however, IC_{50} values are not affected seriously.