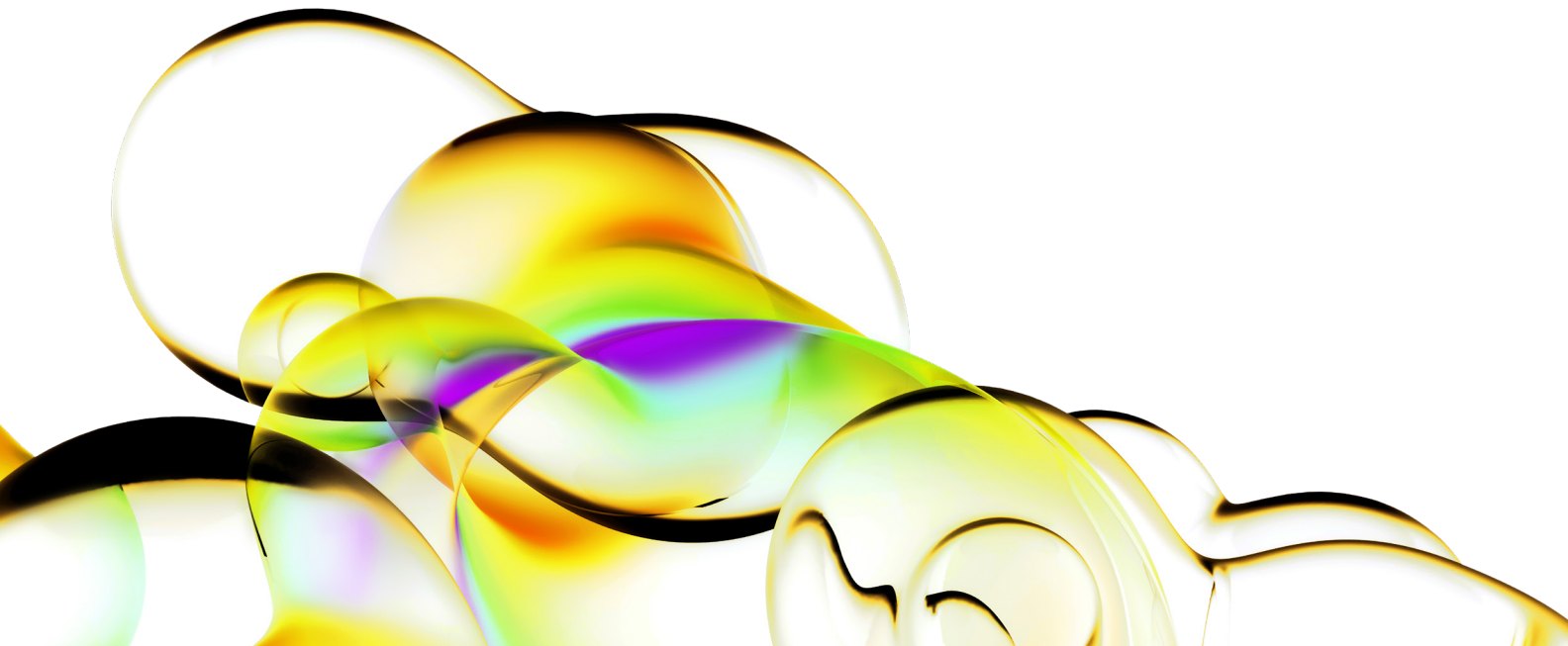
EPIgeneous bromodomain assay: BRD2(1)

Abstract

Bromodomains (BRDs) are protein interaction modules that specifically recognize epsilone-N-lysine acetylation motifs, a key event in the reading process of epigenetic marks. Bromodomain BRD2(1) assay measures the interaction of BRD2(1) with [Lys(5,8,12,16)Ac] H4(1-21) peptide and allows interaction inhibitor study.

This HTRF assay uses a BRD2(1), GST-tag bromodomain protein, [Lys(5,8,12,16) Ac] H4(1-21) biotinylated peptide, and two HTRF detection reagents: donor crypate labeled anti GST antibody and red acceptor conjugated streptavidin. HTRF signal is proportional to the amount of BRD2(1), GST-tag/[Lys(5,8,12,16) Ac] H4(1-21)-biotin peptide in interaction.

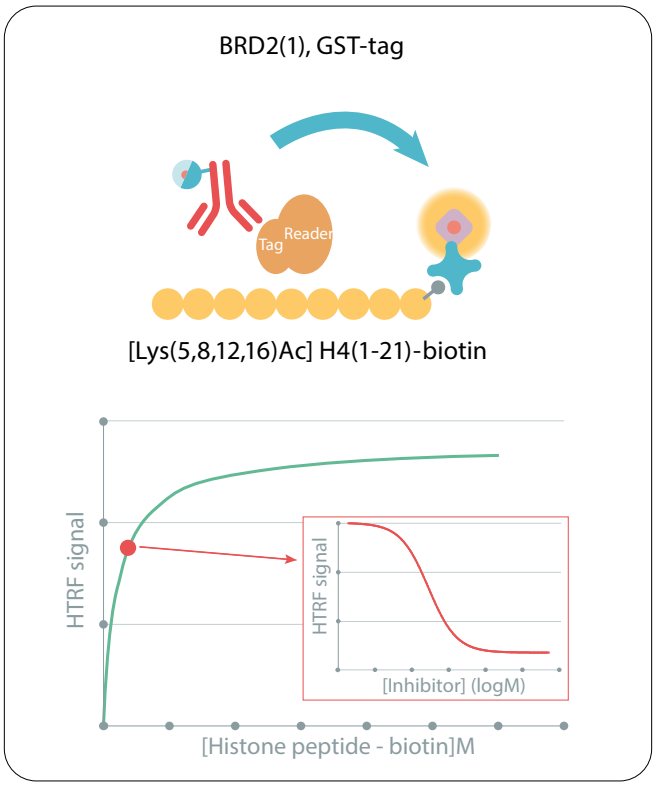
Bromodomain	BRD2(1), GST-tag [BRD2(71-194); RING; RNF3; Bromodomain containing 2]
Histone peptide	[[Lys(5,8,12,16)Ac] H4(1-21)-biotin SGRG-K(Ac)-GG-K(Ac)-GLG-K(Ac)-GGA-K(Ac)-RHRKVGG-K(Biotin)
Detection reagents	EPIgeneous™ Binding Domain Kit A



BRD2(1)/Histone peptide interaction assay and reagents

Reagent	Provider	Reference
EPIgeneous Binding Domain Kit A	Revvity	# 62BDAPEG
BRD2(1), GST-Tag	Reaction Biology	# RD-11-156
[Lys(5,8,12,16)Ac] H4(1-21)-biotin	AnaSpec	# 64989
[Lys(5,8,12,16)Ac] H4(1-25)	AnaSpec	# AS-65421
(+)-JQ1	Tocris	# 4499

Data shown on this application note has been obtained using Greiner # 784075, 384-well white microplates.



Assay protocol

- Dilute the anti GST-Donor Ab 50-fold with Binding Domain Detection Buffer #1 to obtain the working solution ready to be dispensed.
- The peptide-biotin / streptavidin-acceptor ratio must be equal to 8/1 final in the well (e.g. Peptide-biotin used at 4 nM final in the well, SA-Acceptor must be used at 0.5 nM final in the well). Prepare the SA-Acceptor solution in Binding Domain Detection Buffer #1 to get a 4X working solution depending on the final optimal concentration in the well.

Prepare working solutions of protein and biotin-peptide in assay buffer just prior to use.

- We recommend using the GST-tagged binding domain at 5 nM final concentration in the well. Prepare the working solution at 5X depending on the final concentration in the well in Binding Domain diluent buffer (here 25 nM).
- Prepare the peptide-biotin at optimal concentration (referenced in table Optimal experimental conditions) in Binding Domain Diluent Buffer to get a 5X working solution depending on the final optimal concentration in the well.
- Prepare supplemented Binding Domain Diluent Buffer with DMSO to get a constant percentage throughout the inhibitor titration. Dilute the compound in this solution to get a 10X working solution depending on final concentration in the well.

DMSO may act as an inhibitor of GST-binding domain and the biotinylated peptide interaction. This can lead to a decrease of the assay window as DMSO % increases. We recommend the use of compatible DMSO % (See table "Optimal experimental conditions" for DMSO tolerance associated with BRD2(1)).

- Add to a 384-well small volume plate in the following order:
  - 4 µL of BRD2(1), GST-tag (5X)
  - 2 µL of assay buffer (w/ or w/o DMSO)
  - 4 µL of [Lys(5,8,12,16)Ac] H4(1-21)-biotin (5X)
  - 5 µL of SA-Acceptor (4X)
  - 5 µL of anti GST-Donor Ab (4X) 5 µL of anti GST-Donor Ab (4X)
- Cover the plate with a plate sealer and incubate 3h at room temperature.
- *Remark: Signal remains stable after Over Night incubation.*
- Remove plate sealer and read fluorescence emission at 665 nM and 620 nM wavelengths on an HTRF compatible reader.

	Peptide titration		Test of inhibitors		
	Positive signal	Negative control	Inhibitor	Positive control	Negative control
BRD2(1), GST-TAG	4 µL	-	4 µL	4 µL	-
Inhibitor	-	-	2 µL	-	-
Binding domain diluent buffer	2 µL	6 µL	-	2 µL	6 µL
Biotin-peptide	4 µL	4 µL	2 µL	4 µL	4 µL
Streptavidin-acceptor	5 µL	5 µL	5 µL	5 µL	5 µL
Anti GST-donor AB	5 µL	5 µL	5 µL	5 µL	5 µL

Optimal experimental conditions

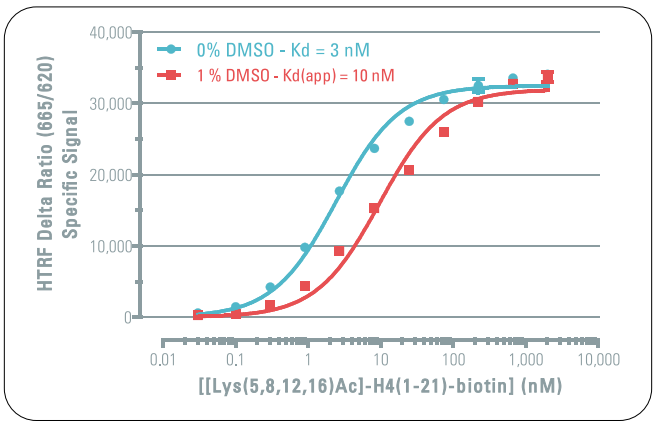
Binding domain	Recommended peptide concentration (Final in the well)	DMSO tolerance
BRD2(1)	0 - 1% DMSO: 3 nM	0 - 1%

Data reduction

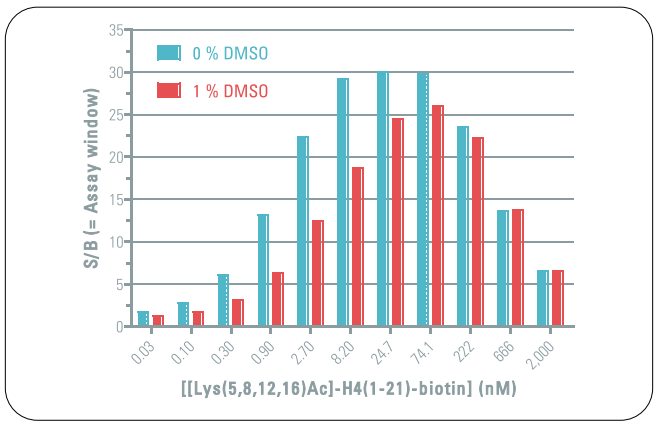
- The TR-FRET signal is treated as HTRF Ratio = Acceptor signal (665 nM) / Donor Signal (620 nM) x 10^4
- HTRF Delta Ratio = Ratio (Positive) – Ratio (Negative) where Negative control is performed without reader-protein.
- Assay window = S/B = Ratio (Positive) / Ratio (Negative)

Results

1. Peptide-biotin titration



2. DMSO effect on assay window



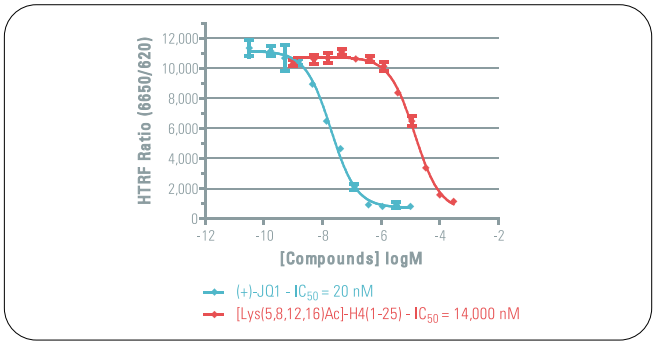
Measurement of BRD2(1) / histone H4 peptide interaction and DMSO effect

The GST-BRD2(1) concentration was fixed at 5 nM while the peptide-biotin was serially diluted. The HTRF Delta Ratio is proportional to the specific interaction measured between GST-BRD2(1) and [Lys(5,8,12,16)Ac]-H4(1-21)-biotin peptide. The 3 nM Kd value is determined from this experiment using a one-site specific binding regression. A shift of apparent Kd is observed while DMSO% increases. This is due to the competitive inhibitor nature of the DMSO on the BRD2(1)/H4 peptide interaction (already described: Philpott et al. Mol. BioSyst., 2011).

Selection of optimal peptide-biotin concentration depending on DMSO % used

Due to the competitive nature of DMSO, the assay window decreases as the DMSO percentage increases. The assay window can then be recovered by increasing the peptide-biotin concentration. The optimal peptide-biotin concentration is selected (between real Kd and EC_{100} obtained on the titration without DMSO) with a compromise between assay window and assay sensitivity for inhibitor studies. Note that the higher the peptide-biotin concentration, the higher the inhibitor IC_{50}. For further study of inhibitors, 1% DMSO and 3 nM peptidebiotin conditions were selected.

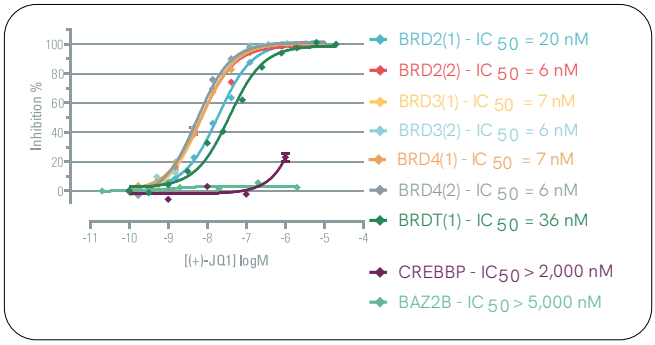
3. Inhibitor titration



BRD2(1) HTRF inhibition assay was validated using reference inhibitors

The HTRF assay was performed using 3 nM peptide-biotin, 5 nM GST-BRD2(1) and 1% DMSO set constant throughout the inhibitor titration. The IC_{50} of (+)-JQ1 and H4 tetra-acetylated peptide are in good agreement with published data (Philpott et al. Mol. BioSyst., 2011/Filippakopoulos and Knapp, FEBS Letters).

4. Compound profiling



Compound selectivity can be assessed over a broad range of validated reader domain assays.

(+)-JQ1 compound was profiled on the BET bromodomain family, CREBBP and BAZ2B bromodomains. As already described (Filippakopoulos et al. Nature 2010), (+)-JQ1 is a non selective inhibitor over the BET family but displays selectivity over non BET bromodomains (CREBBP and BAZ2B).

Related information

Enabling epigenetics studies from HTS to SAR: a novel HTRF® platform to identify and characterize reader domain inhibitors

Roux T, Badol M, Douayry N, Sergeant L, Trinquet E, Degorce F, Milhas S, Betzi S, Derviaux C, Eydoux C, Letienne J, Lugari A, Collette Y, Guillemot J-C, Morelli X. - Revvity Codolet, France | CRCM, CNRS UMR7258, INSERM U1068, AMU UM105, Institut Paoli-Calmettes, Marseille, France | AFMB, UMR7257, Univ. Aix Marseille-CNRS, Marseille, France

How do HTRF® epigenetic binding domain assays perform compared to other technologies?

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