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

Thomas Roux, Najim Douayry, Laurent Sergeant, Eric Trinquet, and François Degorce
Cisbio Bioassays, Codolet, France.

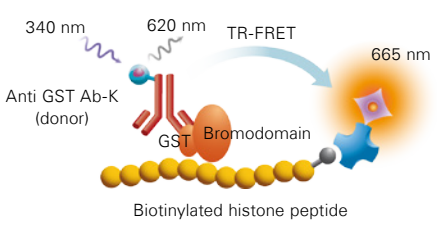
INTRODUCTION Over the past several years, significant effort has been made in drug discovery to identify potent and selective inhibitors of epigenetic targets. Proteins in this target class are classified into readers, writers, and erasers of marks on histones or other nuclear proteins and DNA. By regulating a combination of post-translational marks, these proteins keep tight control over gene expression. Their deregulation has been linked with the development of various diseases, particularly in oncology.

Assay platform based on HTRF technology enable the discovery and characterization of new reader domain inhibitors. In this study, HTRF binding domain assay performance was benchmarked against two other commercially-available assays: Cayman Chemical TR-FRET and AlphaScreen® technology bead-based assays. This poster provides comparative data on the BRD4(1) bromodomain assay.

ASSAY PRINCIPLES

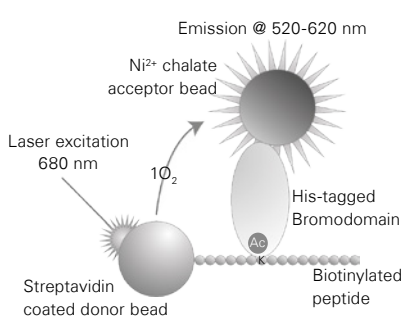
The three assays are designed to measure the interaction between reader domain protein BRD4(1) and modified lysine residues of the N–terminal tails of histones H4. This enables rapid characterization of interaction inhibitors in a high throughput format (384 low-volume plate, 20 µL assay volume).

A. HTRF Assay



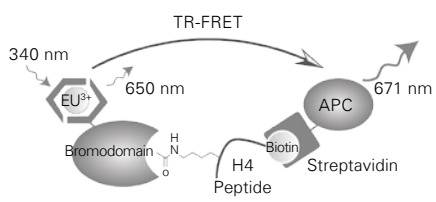
Protocol: following kit instructions

B. AlphaScreen Assay



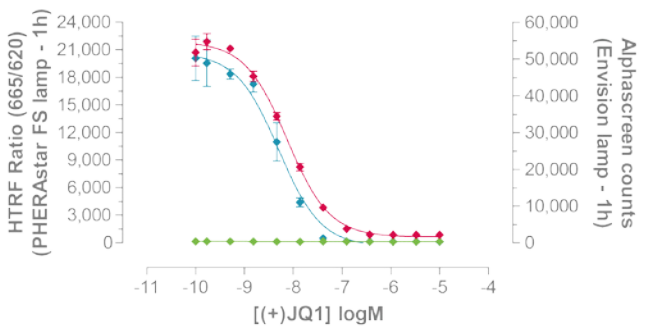
Protocol: as in publication (1), because no instructions could be found on the supplier's website for the BRD4(1) assay. Peptide-biotin concentration was optimized to achieve approximately the same IC_{50} inhibitor as with the HTRF assay.

C. Cayman Chemical TR-FRET Assay



Protocol: following supplier's instructions

LESS PROTEIN REQUIRED

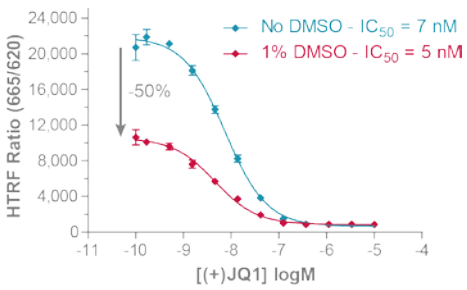


	[BRD4]	IC_{50}
HTRF	5 nM	7 nM
AlphaScreen	50 nM	5 nM
AlphaScreen	5 nM	N.D.

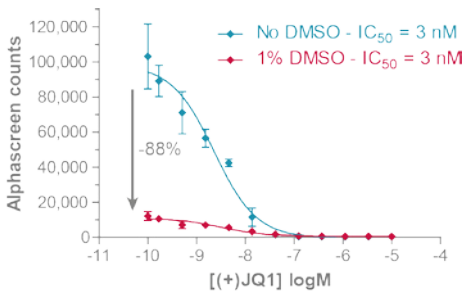
- Both assays display the expected IC_{50} for the reference inhibitor, but the AlphaScreen assay requires 50 nM BRD4(1) protein while the HTRF assay needs 10 time less for similar output.
- BRD4(1) can be decreased down to 1 nM with HTRF assays (data not shown).

BETTER TOLERANCE TO DMSO

A. HTRF assay



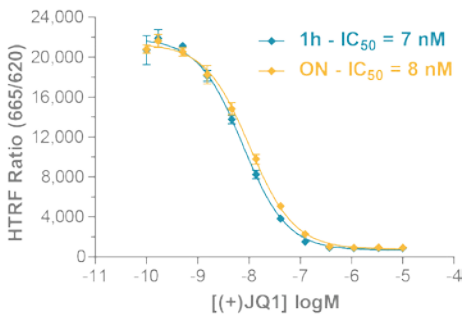
B. AlphaScreen assay



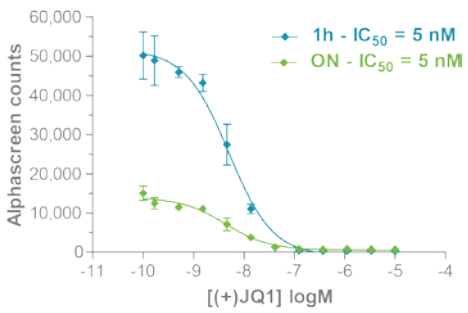
- DMSO is known to be an inhibitor of the BRD4(1) / Histone peptide interaction leading to signal decrease. The HTRF assay displays better DMSO tolerance, as well as significantly tighter intra-assay reproducibility.

INCOMPARABLE SIGNAL STABILITY

A. HTRF assay



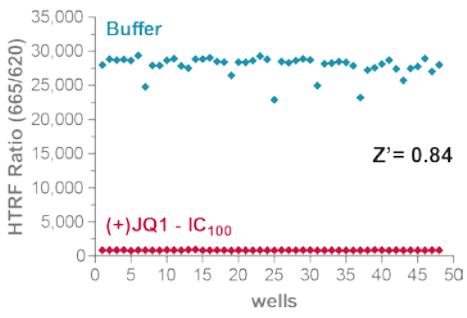
B. AlphaScreen assay



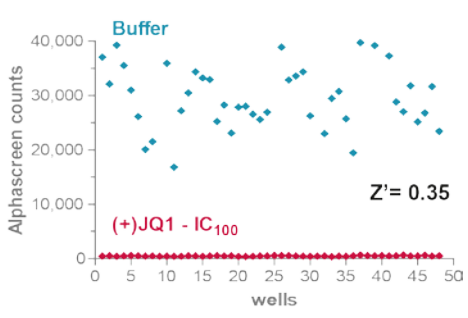
- The HTRF signal remains very stable after overnight incubation, while AlphaScreen counts decrease over time.

CLEAR-CUT ROBUSTNESS

A. HTRF assay



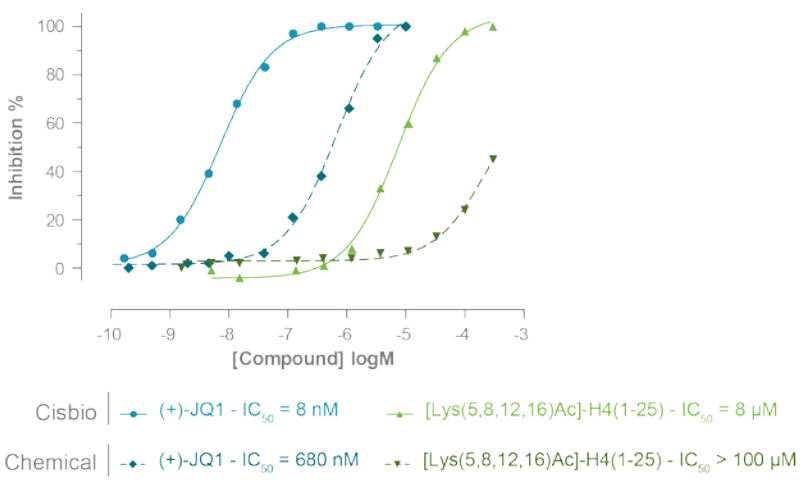
B. AlphaScreen assay



- Homogeneous bead-based technologies, such as AlphaScreen, are typically prone to larger variability, as expressed in this side-by-side robustness test by a significantly degraded z' factor, which challenges the use of such a format for HTS.

Experiments were performed without DMSO.

THE HTRF ASSAY ALSO DISPLAYS BETTER SENSITIVITY



- IC_{50} displayed by the Cisbio (HTRF) assay matches published data: (+)-JQ1 – 50 nM (2) and [Lys(5,8,12,16)Ac]-H4(1-25) – 2.8 µM (3). The Cayman TR-FRET assay shows much higher IC_{50}.
- Both assays display good robustness (assay window and CV%).

CONCLUSION HTRF has been a technology of choice for building protein:protein interaction assays for over two decades. Leveraging this experience, we developed a novel platform to identify and characterize reader domain inhibitors. 28 reader domains from different families have been validated successfully as of this writing(4).

Among these assays, the HTRF BRD4(1) assay was included in a comparison with two other assays using AlphaScreen (Perkin Elmer) or TR-FRET (Cayman Chemical). In both cases the HTRF format provides significantly superior output, notably in terms of robustness, overall cost-effectiveness, and sensitivity.

Key points include:

- Enhanced robustness and better DMSO tolerance with respect to AlphaScreen, making this new platform ideally suited for HTS of binding domain targets.
- Lower consumption of protein than with the equivalent AlphaScreen assay, providing a more cost-effective solution for screening.
- Better assay sensitivity when compared to the Cayman Chemical TR-FRET kit, enabling the identification of all inhibitors including those with low affinity.

REFERENCES

(1) Philpott et al. Mol. BioSyst., 2011, 7, 2899-2908
(2) Filippakopoulos et al, Nature 468 (2010) 1067–1073
(3) Filippakopoulos and Knapp, FEBS Letters 586 (2012) 2692–2704
(4) Roux et al. Miptec 2014. http://www.cisbio.com/epigenetic-binding-domain

Europe and other countries +33(0)466 796 705 **U.S. and Canada** 1-888-963-4567 **China** +86 21 5018 9880 **Japan** +81-(0)43-306-8712
www.cisbio.com