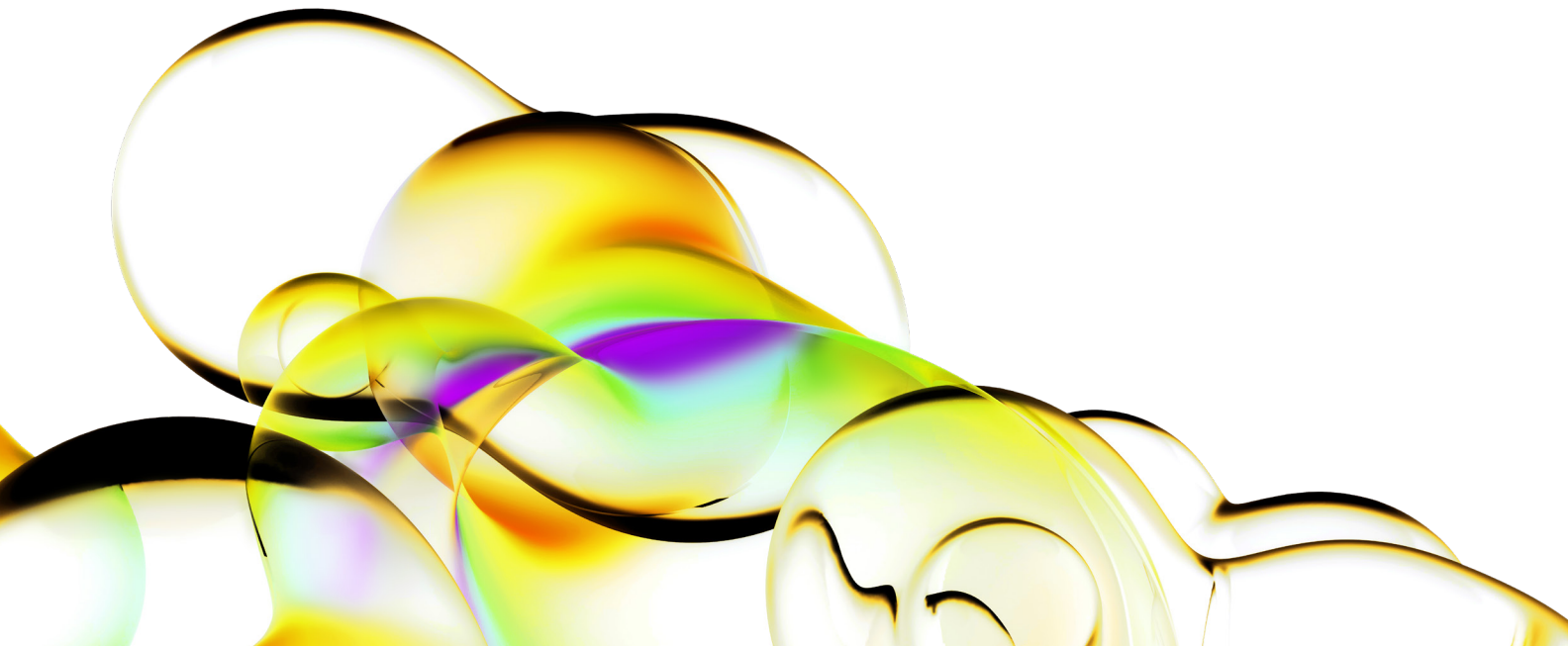
# EPIgeneous chromodomain assay CBX1


## Abstract

Chromodomains are protein interaction modules that recognize methylated lysine motifs, a key event in the reading process of epigenetic marks. Chromodomain CBX1 assay measures the interaction of CBX1 with [Lys(9)Me3] H3(1-21) peptide and allows interaction inhibitor study.

This HTRF® assay uses a CBX1, GSTtag chromodomain protein, [Lys(9) Me3] H3(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 CBX1, GST-tag/[Lys(9)Me3] H3(1-21)-biotin peptide in interaction.

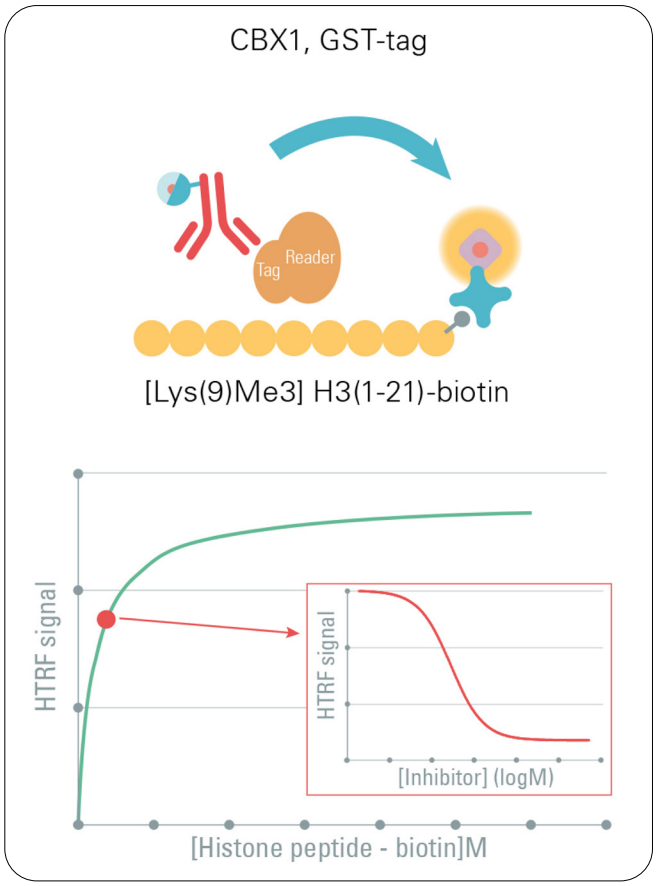
| | |
|---|---|
| Bromodomain | CBX1, GST-tag<br>[CBX1(2-185) ; HP1-beta] |
| Histone peptide | [Lys(9)Me3] H3(1-21)-biotin<br>ARTKQTAR-K(Me3) -STGGK APRKQLAGGK( Biotin) |
| Detection reagents | EPIgeneous™ Binding Domain Kit A |

## CBX1/Histone peptide interaction assay and reagents

| Reagent | Provider | Reference |
|---|---|---|
| EPIgeneous Binding Domain Kit A | Revvity | # 62BDAPEG |
| CBX1, GST-Tag | BPS Bioscience | # 55009 |
| [Lys(9)Me3] H3 (1-21) -biotin | AnaSpec | # 64360 |

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 CBX1).*

- Add to a 384-well small volume plate in the following order:
  - 4 µL of CBX1, GST-tag (5X)
  - 2 µL of assay buffer (w/ or w/o DMSO)
  - 4 µL of [Lys(9)Me3] H3(1-21)-biotin (5X)
  - 5 µL of SA-Acceptor (4X)
  - 5 µL of anti GST-Donor Ab (4X)
- Cover the plate with a plate sealer and incubate 1h 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 |
| CBX1, 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

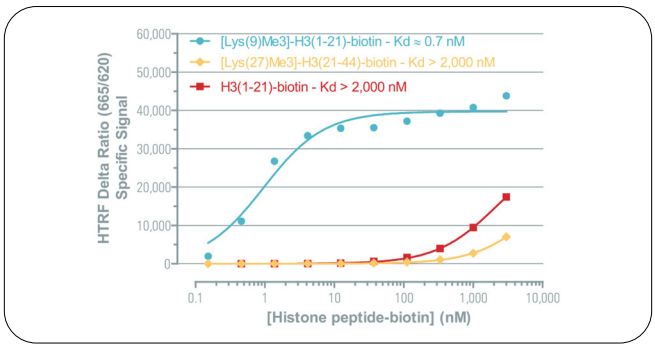
| Binding domain | Recommended peptide concentration (Final in the well) | DMSO tolerance |
|---|---|---|
| CBX1 | 0 - 4% DMSO: 1 nM | 0 - 4% |

### Data reduction

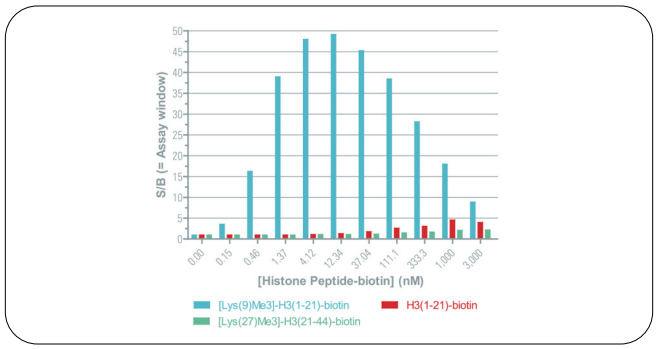
- The TR-FRET signal is treated as HTRF Ratio = Acceptor signal (665 nM)/Donor Signal (620 nM) x $10^4$
- HTRF Delta Ratio = Ratio (Positive) – Ratio (Negative) where Negative control is performed without reader-protein.
- Assay window = S/B = Ratio (Positive)/Ratio (Negative)

## Results

### 1. Peptide-biotin titration




### 2. Assay window with various peptide-biotin



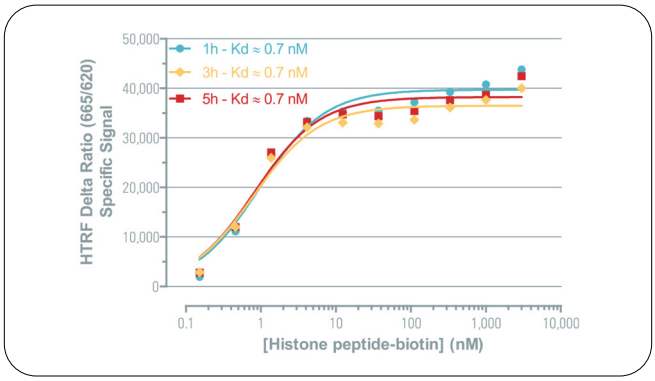

### Measurement of CBX1 / histone H3 peptide interaction and peptide selection

The GST-CBX1 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-CBX1 and peptide-biotin. The 0.7nM Kd value for [Lys(9)Me3]-H3(1-21)-biotin peptide is determined from this experiment using a one-site specific binding regression.
As expected, unmodified histone peptide-biotin and K27Me3 methylated peptide display very low specific binding compared to K9Me3 peptide (Jacobs et al. EMBO, 2001 and Kaustov et al. Jbc, 2011).

### Selection of optimal peptide-biotin and concentration

The [Lys(9)Me3]-H3(1-21)-biotin peptide which gives the best results was selected at 1nM for inhibitor titration. Note that the higher the peptide-biotin concentration, the higher the inhibitor $IC_{50}$.

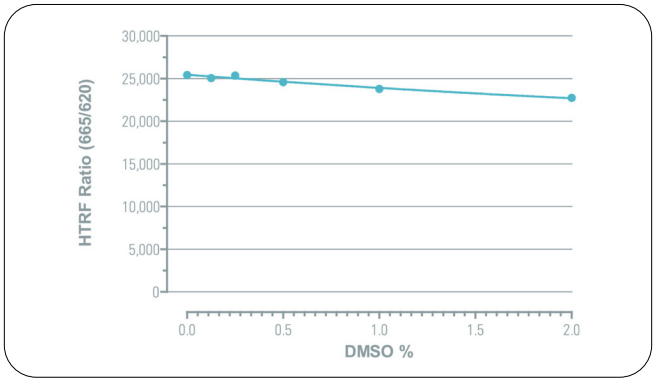
### 3. Kinetic of peptide-biotin binding




**Measurement of CBX1 / histone H3 peptide interaction kinetic**

The GST-CBX1 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-CBX1 and [Lys(9)Me3] H3(1-21)-biotin peptide. Equilibrium of kinetic binding of peptide-biotin on bromodomain is reached at 1h.

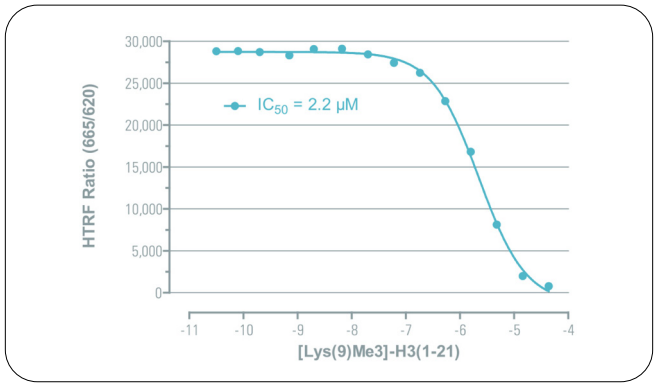
### 4. DMSO effect




**CBX1 HTRF assay displays very good DMSO tolerance**

The HTRF assay was performed using 2 nM peptide-biotin, 5 nM GST-CBX1 and DMSO ranging from 0 to 2%.
No dmajor DMSO effect is observed on signal.

### 5. Inhibitor titration




**CBX1 HTRF inhibition assay was validated using reference inhibitors**

The HTRF assay was performed using 1 nM peptide-biotin, 5 nM GST-CBX1 and 1% DMSO set constant throughout the inhibitor titration. The $IC_{50}$ of [Lys(9)Me3]-H3(1-21) peptide is in good agreement with published data (Kaustov et al. Jbc, 2011 - 5µM)

## 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?

*Thomas Roux, Najim Douayry, Laurent Sergeant, François Degorce and Eric Trinquet. Revvity Codolet, France*

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