EPIGENEOUS TUDOR DOMAIN ASSAY: UHRF1

TECHNICAL NOTE

ABSTRACT Tudor domains are protein interaction modules that recognize methylated lysine motifs, a key event in the reading process of epigenetic marks. Tudor domain UHRF1 assay measures the interaction of UHRF1 with [Lys(9)Me3] H3(1-21) peptide and allows interaction inhibitor study.

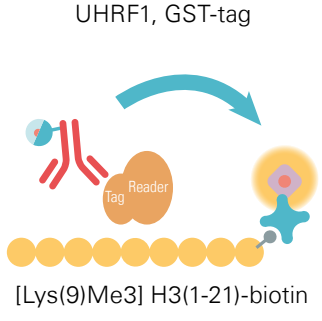
This HTRF assay uses a UHRF1, GST-tag tudor domain protein, [Lys(9)Me3] H3(1-21) biotinylated peptide, and two HTRF detection reagents: donor crypate labeled anti GST antibody and red acceptor conjugated streptavidin. HTRF signal is proportional to the amount of UHRF1, GST-tag / [Lys(9) Me3] H3(1-21)-biotin peptide in interaction.

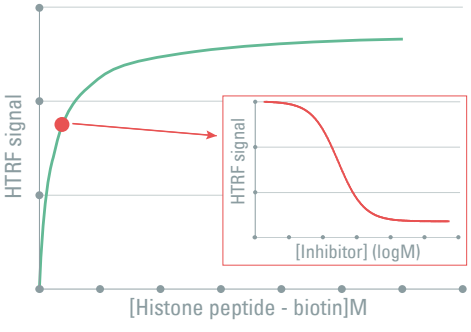
Tudor domain	UHRF1, GST-tag [UHRF1(108-286) ; RNF106]
Histone peptide	[Lys(9)Me3] H3(1-21)-biotin ARTKQTAR-K(Me3)-STGGKAPRKQLA-GGK(Biotin)
Detection reagents	EPIgeneous Binding Domain Kit B

UHRF1 / HISTONE PEPTIDE INTERACTION ASSAY AND REAGENTS

REAGENT	PROVIDER	REFERENCE
EPIgeneous Binding Domain Kit B	Cisbio Bioassays	# 62BDBPEG
UHRF1, GST-Tag	BPS Bioscience	# 55003
[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. For more information on the white plates, please visit www.cisbio.com/htrf-microplate-recommendations





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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 5nM 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 UHRF1).

- Add to a 384-well small volume plate in the following order:
  - 4 µL of UHRF1, 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 3h at room temperature.
- *Remark: Signal remains stable after Over Night incubation.*
- Remove plate sealer and read fluorescence emission at 665nm and 620nm wavelengths on an HTRF compatible reader *.

	PEPTIDE TITRATION		TEST OF INHIBITORS		
	POSITIVE SIGNAL	NEGATIVE CONTROL	INHIBITOR	POSITIVE CONTROL	NEGATIVE CONTROL
UHRF1, 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

* For more information on HTRF compatible reader, please visit www.cisbio.com/htrf-compatible-readers.

OPTIMAL EXPERIMENTAL CONDITIONS

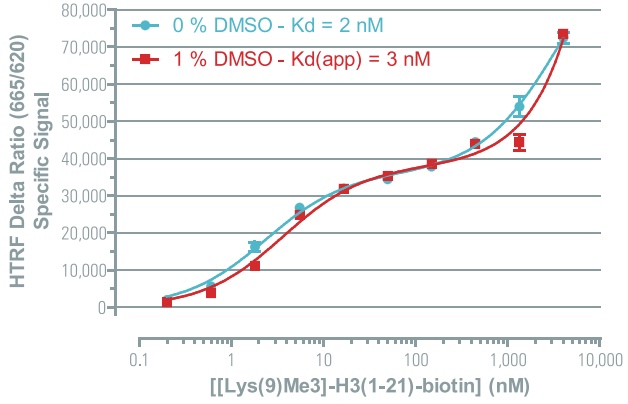
BINDING DOMAIN	RECOMMENDED PEPTIDE CONCENTRATION (FINAL IN THE WELL)	DMSO TOLERANCE
UHRF1	0 - 1% DMSO: 6 nM	0 - 1%

DATA REDUCTION

- The TR-FRET signal is treated as HTRF Ratio = Acceptor signal (665nm) / Donor Signal (620nm) 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

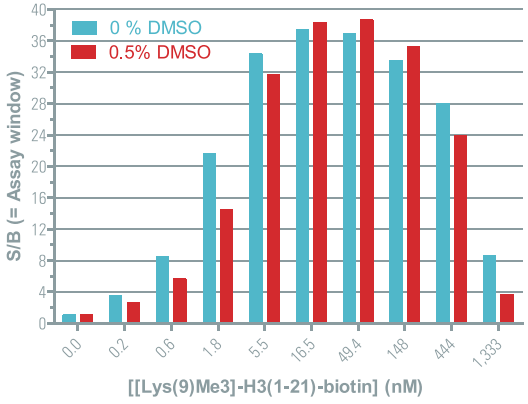
1. PEPTIDE-BIOTIN TITRATION



Measurement of UHRF1 / histone H3 peptide interaction and peptide selection

The GST-UHRF1 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-UHRF1 and peptide-biotin. The 2nM Kd value is determined from this experiment using a two-site specific binding regression. A slight shift of apparent Kd is observed when DMSO% increases.

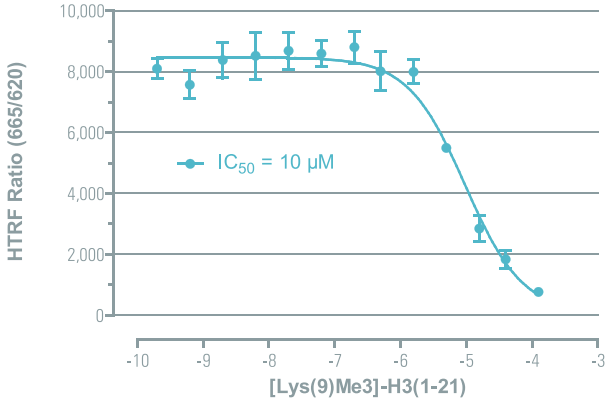
2. DMSO EFFECT ON ASSAY WINDOW



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

The assay window slightly 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 EC100 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 IC50. For further study of inhibitors, 1% DMSO and 6nM peptide-biotin conditions are recommended.

3. INHIBITOR TITRATION



UHRF1 HTRF inhibition assay was validated using reference inhibitors

The HTRF assay was performed using 4 nM peptide-biotin, 5 nM GST-UHRF1 and 1% DMSO set constant throughout the inhibitor titration. The IC50 of [Lys(9)Me3]-H3(1-21) peptide is in good agreement with published data (Rothbart et al. Nat Struct Mol Biol, 2012 - 2µM)

For more information, please visit us at www.cisbio.com/epigenetic-binding-domain

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. - Cisbio Bioassays 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.
Cisbio Bioassays Codolet, France

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