



# HTRF® HDAC2 HISTONE H3K4 DEACETYLATION ASSAY

## APPLICATION NOTE

**ABSTRACT** HDAC2 Histone H3K4 deacetylation assay that measures the deacetylation of a biotinylated histone H3(1-21) peptide at lysine 4.

The HTRF HDAC2 Histone H3K4 deacetylation assay uses a H3(1-21) lysine 4 acetylated biotinylated peptide (substrate), a Eu<sup>3+</sup>-cryptate labeled anti-H3K4me0 detection antibody and XL665-conjugated Streptavidin (SA-XL665).

The assay is performed in a single well and run in two steps: the enzymatic step and the detection step. HTRF signal is proportional to the concentration of Unacetylated H3(1-21) peptide. The assays within this technical note were performed in a 384-well plate in a 20 µL final volume.

Enzyme	HDAC2
Substrate	H3(1-21)K4Ac-biotin ART-K(Ac)-QTARKSTGG- KAPRKQLA-GGK(biotin)
Detection Antibody	Anti-H3K4me0-Eu(K)

## HDAC2 HISTONE H3K4 DEACETYLATION ASSAY AND REAGENTS

H3K4me0-Eu(K) Ab.	Cisbio Bioassays	# 61KA0KAE
Streptavidin XL-665	Cisbio Bioassays	# 610SAXLA
Detection buffer	Cisbio Bioassays	# 62SDBRDD
HDAC2	BPS Bioscience	# 50002
Histone H3(1-21) lysine 4 acetylated biotinylated peptide	AnaSpec	# AS-65207
SAHA	R&D Systems	# 4652
Trichostatin A (TSA)	R&D Systems	# 1406
Enzymatic buffer	50mM Tris-HCl pH 8.0, 1mM DTT, 0.01% Tween, 0.01% BSA	

Data shown on this application note has been obtained using Greiner # 784075, 384-well white microplates. For more information on the white plates we recommend, please visit [www.cisbio.com/htrf-microplate-recommendations](http://www.cisbio.com/htrf-microplate-recommendations).

### Enzymatic step



H3(1-21)K4Ac-biotin

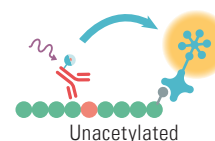


HDAC2



Unacetylated

### Detection step



Unacetylated

## ASSAY PROTOCOL

### ENZYMATIC STEP

- Prepare working solutions of enzyme, peptide substrate, cofactors and inhibitor in enzymatic buffer just prior to use.
- Add to a 384-well small volume plate in the following order:
  - 4  $\mu$ L of inhibitor (2.5X) or enzymatic buffer
  - 2  $\mu$ L of HDAC2 enzyme (5X)
  - Incubate for 5 min at room temperature
  - 4  $\mu$ L of H3(1-21)K4Ac-biotin peptide mixture (2.5X)
- Cover the plate with a plate sealer and incubate at room temperature.

### DETECTION STEP

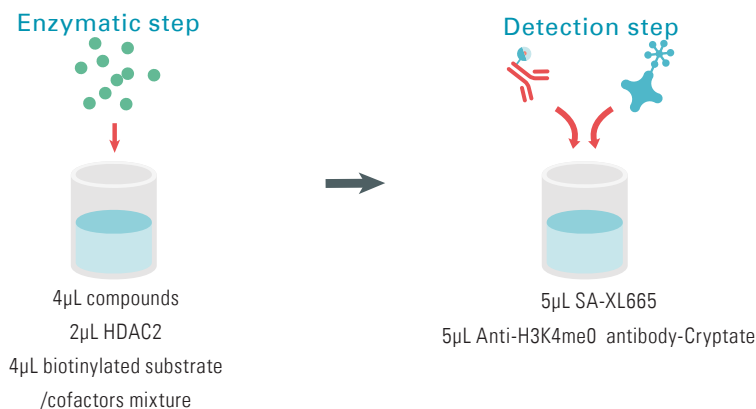
- The peptide-biotin / streptavidin-XL665 ratio must be equal to 4/1 final in the well (e.g. if peptide-biotin used at 4 nM final in the well, SA-XL665 must be used at 1 nM final in the well)
- Prepare detection mixture containing the anti-H3K4me0-Eu(K) 2X according to the product datasheet recommended final concentration and SA-XL665 at 2X the final concentration (hence 0.5X the final concentration of peptide-biotin in the well) in Detection buffer.
- Caution: Adjust SA-XL665 concentration according to peptide-biotin concentration used.
- Add 10  $\mu$ L of detection mixture (2X) to the plate.
- Cover the plate with a plate sealer and incubate 1h at room temperature.
- Remove plate sealer and read fluorescence emission at 665nm and 620nm wavelengths on an HTRF compatible reader.

$$\text{HTRF Ratio} = (665\text{nm}/620\text{nm}) \times 10^4$$

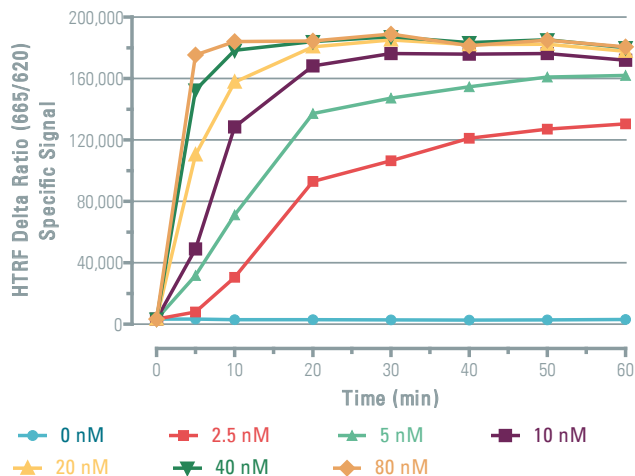
$$\text{Delta Ratio} = \text{Sample Ratio} - \text{Ratio negative}$$

### DISTRIBUTION: ENZYME INHIBITION STUDY

	ENZYMATIC STEP				DETECTION STEP	
	ENZYMATIC BUFFER	INHIBITOR	HDAC2	COFACTOR/SUBSTRATE MIXTURE	CRYPTATE-Ab	SA-XL 665
<b>SAMPLE</b>	-	4 $\mu$ L	2 $\mu$ L	4 $\mu$ L	5 $\mu$ L	5 $\mu$ L
<b>POSITIVE CONTROL</b>	4 $\mu$ L	-	2 $\mu$ L	4 $\mu$ L	5 $\mu$ L	5 $\mu$ L
<b>NEGATIVE CONTROL</b>	6 $\mu$ L	-	-	4 $\mu$ L	5 $\mu$ L	5 $\mu$ L

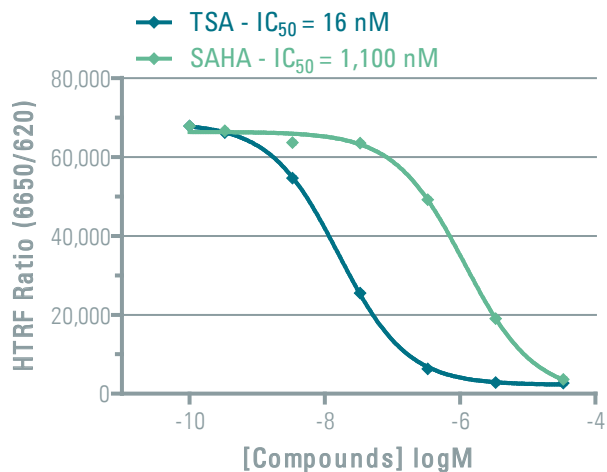


## 1. TIME COURSE AND ENZYME TITRATION



This step allows the optimal enzyme concentration and enzyme reaction time to be determined. Human recombinant HDAC2 was serially diluted to the concentrations indicated in the figure (HDAC2; 2.5, 5, 10, 20, 40 ng/well), and the assay was carried out with 1,000 nM biotinylated H3(1-21)K4Ac peptide substrate. Enzyme kinetics depends on the HDAC2 specific activity and substrate concentrations. The enzymatic reaction was carried out at RT and then stopped by adding H3K4me0-K Ab and SA-XL665 (detection reagents) after each time point (5, 10, 20, 30, 40, 50, 60 min). A 30 min reaction time using 2nM HDAC2 was selected for other experiments.

## 2. ENZYME INHIBITION



HDAC2 H3K4 deacetylation inhibitor assay was validated by measuring the activity of reference inhibitors. This assay was performed using 2 nM HDAC2. Serial dilutions of inhibitors ranged from 0.1 to 33,000 nM and were pre-incubated for 5 min with HDAC2 enzyme. Enzymatic reaction was initiated by the addition of 1,000 nM biotinylated H3(1-21)K4Ac peptide substrate. The enzyme reaction was stopped with the detection conjugates after 30 min incubation at RT. IC<sub>50</sub> values were calculated from inhibition curves.

For more information, please visit us at [www.cisbio.com/epigenetic-toolbox-reagents](http://www.cisbio.com/epigenetic-toolbox-reagents)

## RELATED ARTICLES

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*Roux T, Douayry N, Junique S, Sergeant L, Donsimoni G, Bourrier E, Trinquet E, LaRose R, Degorce F. - EpiCongress 2013, Boston, MA, USA.*

High-Throughput, Homogeneous Histone Demethylase JARID1A, and JARID1C Enzymatic applications with HTRF Technology.

*Adachi K, Tokuda C, Roux T, Trinquet E, Degorce F - Miptec 2013, Basel, Switzerland.*

High-Throughput, Homogeneous Histone H3 Methyltransferase, (HMT) and Demethylase (HDM) Enzyme Assays using HTRF®, Technology: G9a H3K27di-methylation assay example.

*Roux T, Adachi K, Tokuda C, Verdi J, Junique S, Trinquet E, Gonzalez-Moya A, Degorce F - SLAS 2013, Orlando, USA.*

High-Throughput, Homogeneous Histone H3 Methyltransferase (HMT) and Demethylase (HDM) Enzyme Assays using HTRF Technology.

*Adachi K, Tokuda C, Chevallier F, Roux T, Gonzalez-Moya A, Degorce F. - Discovery on Target 2012, Boston, MA, USA.*

Development of a panel of HTRF assay reagents for epigenetic targets.

*Chevallier F, Jean A, Raynaldy D, Romier M, Servent F, Tokuda C, Adachi K. - Miptec 2011, Basel, Switzerland.*

Development of G9a (Histone H3K9 methyltransferase) assay using HTRF technology.

*Adachi K, Tokuda C, Chevallier F, Preaudat M. - SBS 2011, Orlando, USA.*

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