# HTRF® HDAC2 HISTONE H3K4 DEACETYLATION ASSAY

TECHNICAL 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 $Eu^{3+}$-cryptate labeled anti-H3K4me0 detection antibody and XL665-conjugated Streptavidin (SA-XL665).

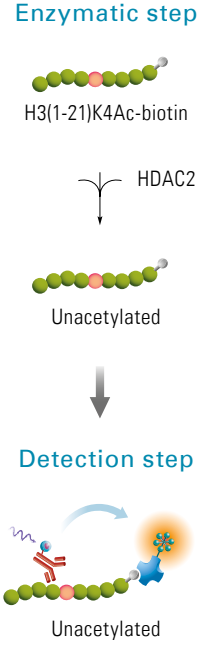
The assay is performed in a single well and run in two steps: the enzymatic step and the detection step. HTRF signal is proportional to the concentration of Unacetylated H3(1-21) peptide. The assays within this technical note were performed in a 384-well plate in a 20 µL final volume.

| | |
|---|---|
| Enzyme | HDAC2 |
| Substrate | H3(1-21)K4Ac-biotin<br>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.

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## 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 µL of inhibitor (2.5X) or enzymatic buffer
  - 2 µL of HDAC2 enzyme (5X)
  - Incubate for 5 min at room temperature
  - 4 µL of H3(1-21)K4Ac-biotin peptide mixture (2.5X)
- Cover the plate with a plate sealer and incubate at room temperature.

### DETECTION STEP

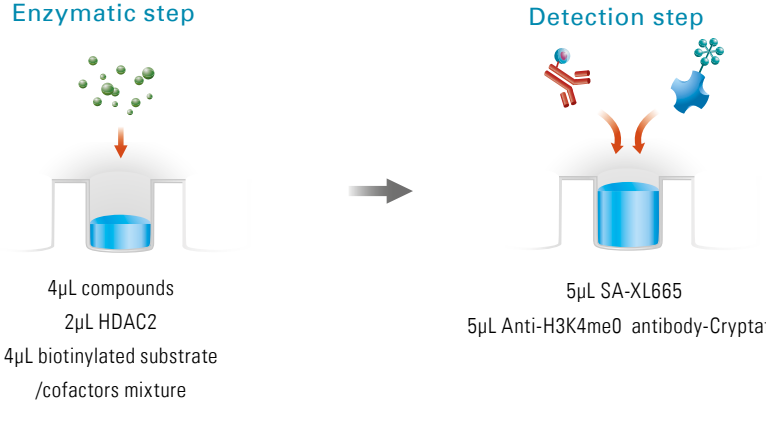
- The peptide-biotin / streptavidin-XL665 ratio must be equal to 4/1 final in the well (e.g. if peptide-biotin used at 4 nM final in the well, SA-XL665 must be used at 1 nM final in the well)
- Prepare detection mixture containing the anti-H3K4me0-Eu(K) 2X according to the product datasheet recommended 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 µL 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.

HTRF Ratio = (665nm/620nm)X$10^4$

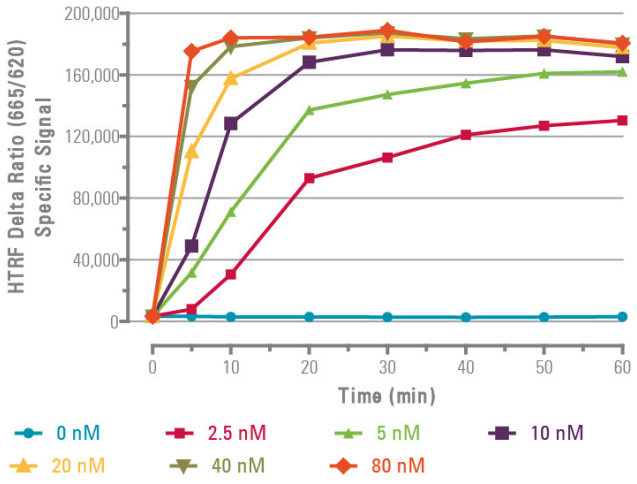
Delta Ratio = Sample Ratio - Ratio negative

### DISTRIBUTION: ENZYME INHIBITION STUDY

| | ENZYMATIC STEP | | | | DETECTION STEP | |
|---|---|---|---|---|---|---|
| | ENZYMATIC BUFFER | INHIBITOR | HDAC2 | COFACTOR/ SUBSTRATE MIXTURE | CRYPTATE-Ab | SA-XL 665 |
| SAMPLE | - | 4 µL | 2 µL | 4 µL | 5 µL | 5 µL |
| POSITIVE CONTROL | 4 µL | - | 2 µL | 4 µL | 5 µL | 5 µL |
| NEGATIVE CONTROL | 6 µL | - | - | 4 µL | 5 µL | 5 µL |

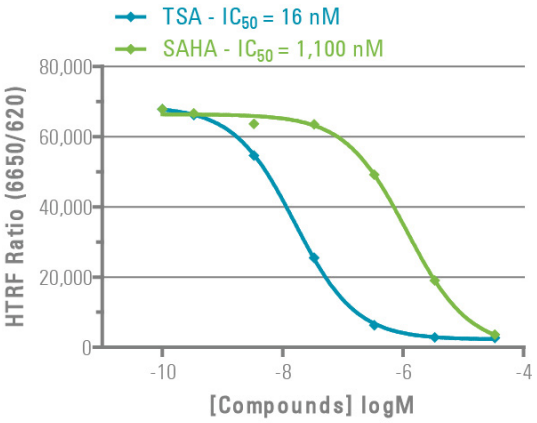


## 1. TIME COURSE AND ENZYME TITRATION




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 dectection conjugates after 30 min incubation at RT. IC50 values were calculated from inhibition curves.

**For more information, please visit us at www.cisbio.com/epigenetic-toolbox-reagents**

## RELATED ARTICLES


EPIgeneousTM Methyltransferase assay: a new HTRF Universal, SAH detection assay to assess methyltransferase activity.
*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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