



HTRF® MLL1/WARD HISTONE H3K4 TRI-METHYLATION ASSAY (me0 → me3)

TECHNICAL NOTE

ABSTRACT MLL1/WARD Histone H3K4 tri-methylation assay measures the trimethylation of a biotinylated histone H3(1-50) peptide at lysine 4.

The HTRF MLL1/WARD Histone H3K4 trimethylation assay uses a H3(1-50) lysine 4 un-methylated biotinylated peptide (substrate), a Eu3+-cryptate labeled anti-H3K4 me3 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 trimethylated H3(1-50) peptide. The assays within this technical note were performed in a 384-well plate in a 20 µL final volume.

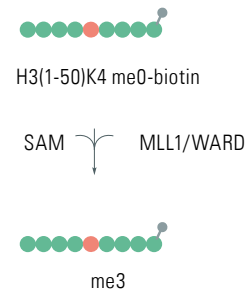
Enzyme	MLL1/WARD
Substrate	H3(1-50)K4 me0-biotin ARTKQTARKSTGG- KAPRKQLATKAARKSA- PATGGVKKPHRYRPGTVAL- REGG-K(Biotin)
Detection Antibody	Anti-H3K4 me3-Eu(K)

MLL1/WARD HISTONE H3K4 TRI-METHYLATION ASSAY AND REAGENTS

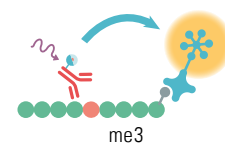
H3K4 me3-Eu(K) Ab.	Cisbio Bioassays	# 61KA3KAE
Streptavidin XL-665	Cisbio Bioassays	# 610SAXLA
Detection buffer	Cisbio Bioassays	# 62SDBRDD
MLL1/WARD	Cayman Chemical	# 10945
Histone H3(1-50) lysine 4 un-methylated biotinylated peptide	AnaSpec	# 65366
S-(5'-Adenosyl)-L-methionine chloride (SAM)	Sigma	# A7007
Sinefungin	Sigma	# S8559
Enzymatic buffer	50 mM Tris-HCl, pH 8.8, 10 mM NaCl, 4 mM DTT, 4 mM MgCl2, 0.01% Tween20	

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 <http://www.htrf.com/htrf-technology/microplate-recommendations>.

Enzymatic step



Detection step



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 μ L of inhibitor (2.5X) or enzymatic buffer
 - 2 μ L of MLL1/WARD enzyme (5X)
 - Incubate for 5 min at room temperature
 - 4 μ L of H3(1-50)K4 me0-biotin peptide/ SAM pre-mixture (2.5X)
- Cover the plate with a plate sealer and incubate at room temperature.

DETECTION STEP

- Prepare detection mixture containing the anti-H3K4 me3-Eu(K) 2X according to the product datasheet recommended final concentration and SA-XL665 at 50 nM in detection buffer. Final concentration of 25 nM for SA-XL665 corresponds to 0.25X the final concentration of peptide substrate.
- Add 10 μ 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.

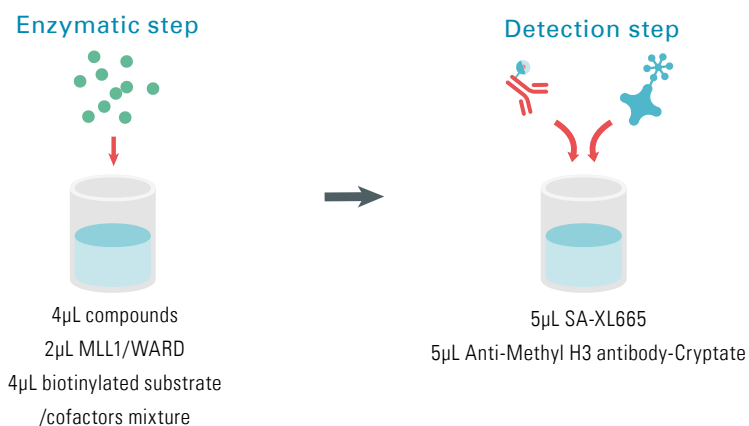
$$\text{HTRF Ratio} = (665\text{nm}/620\text{nm}) \times 10^4$$

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

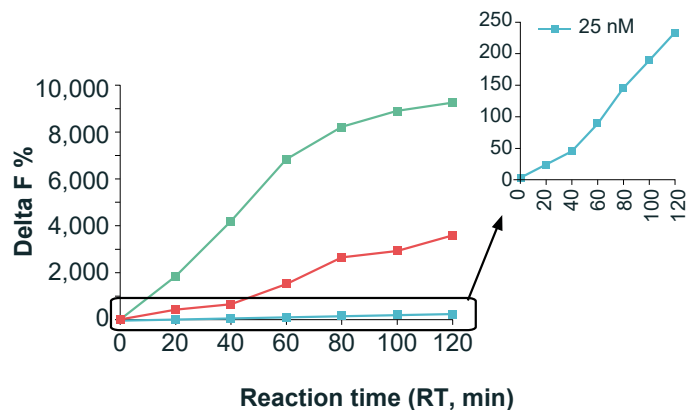
$$\text{Delta F\%} = (\text{Delta Ratio}/\text{Ratio Negative}) \times 100$$

DISTRIBUTION: ENZYME INHIBITION STUDY

	ENZYMATIC STEP				DETECTION STEP	
	ENZYMATIC BUFFER	INHIBITOR	MLL1/WARD	COFACTOR/SUBSTRATE MIXTURE	CRYPTATE-Ab	SA-XL 665
SAMPLE	-	4 μ L	2 μ L	4 μ L	5 μ L	5 μ L
POSITIVE CONTROL	4 μ L	-	2 μ L	4 μ L	5 μ L	5 μ L
NEGATIVE CONTROL	6 μ L	-	-	4 μ L	5 μ L	5 μ L



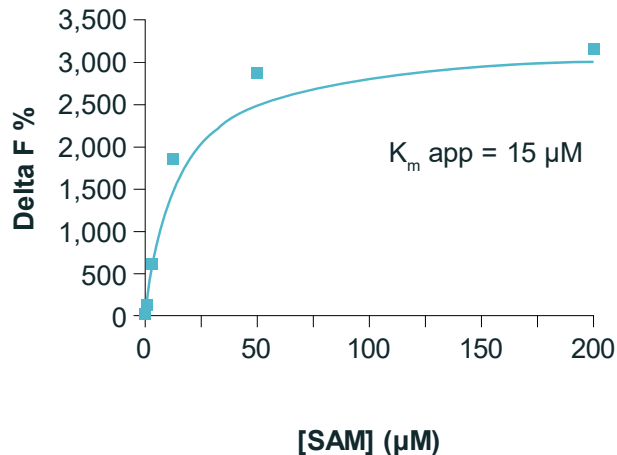
1. TIME COURSE AND ENZYME TITRATION



[MLL1/WARD] — 25 nM — 50 nM — 100 nM

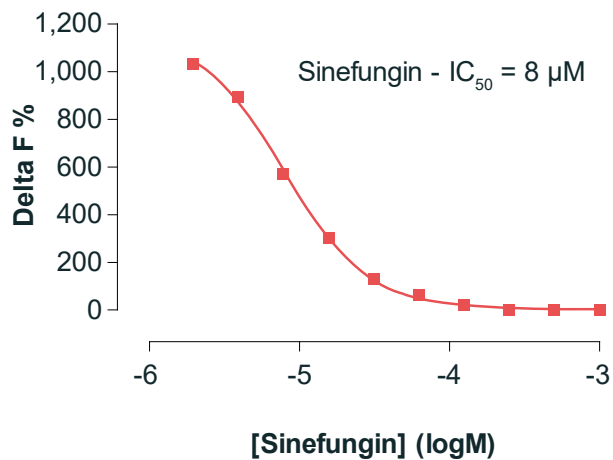
This step allows the optimal enzyme concentration and enzyme reaction time to be determined. Human recombinant MLL1/WARD complex was serially diluted to the concentrations indicated in the figure (25, 50, 100 nM), and the assay was carried out with 200 nM biotinylated H3(1-50)-me0 peptide substrate and 200 μ M SAM. Enzyme kinetics depends on the MLL1 specific activity and substrate concentrations. The enzymatic reaction was carried out at RT and then stopped by adding H3K4me3-K Ab and SA-XL665 (detection reagents) after each time point (20, 40, 60, 80, 100, 120 min). For further experiments, a reaction time of 120 min at RT and 50 nM enzyme complex were selected.

2. SAM TITRATION



This step allows the determination of K_m for SAM. The K_m value was determined with 50 nM MLL1/WARD complex and 200 nM biotinylated H3(1-50)me0 substrate in the enzymatic step. We recommend testing SAM concentrations ranging from 200 μ M to 0.195 μ M (serial dilutions). The enzyme reaction was stopped at the optimal incubation period (RT, 120 min) by adding the detection reagents. The 15 μ M K_m value for SAM was determined from this experiment using a Michaelis-Menten plot.

3. ENZYME INHIBITION



MLL1/WARD H3K4 trimethylation inhibitor assay was validated by measuring the activity of sinefungin inhibitor. This assay was performed using 20 μ M SAM and 50 nM MLL1/WARD complex. Serial dilutions of sinefungin ranged from 1.9 μ M to 1 mM and were pre-incubated for 5 min with MLL1/WARD complex. Enzymatic reaction was initiated by the addition of 200 nM biotinylated H3 (1-50) peptide substrate plus 20 μ M SAM. The enzyme reaction was stopped with the detection reagents after 120 min incubation at RT. IC_{50} value calculated from the inhibition curve was 7.9 μ M.

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

RELATED ARTICLES

EPIgeneous™ 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 H3K-27dimethylation 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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