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).

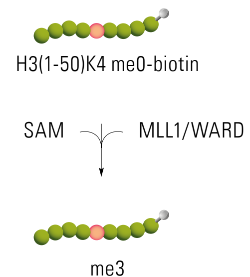
The assay is performed in a single well and run in two steps: the enzymatic step and the detection step. HTRF signal is proportional to the concentration of trimethylated H3(1-50) peptide. The assays within this technical note were performed in a 384-well plate in a 20 µL final volume.

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

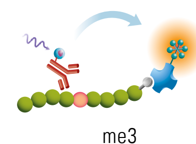
MLL1/WARD HISTONE H3K4 TRI-METHYLATION ASSAY AND REAGENTS

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	

Enzymatic step



Detection step



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.

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INTERACTION IS EVERYTHING

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 MLL1/WARD enzyme (5X)
  - Incubate for 5 min at room temperature
  - 4 µL 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 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)X10^4

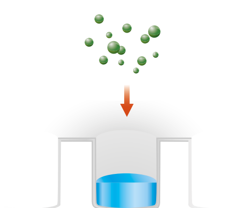
Delta Ratio = Sample Ratio - Ratio negative

Delta F% = (Delta Ratio/Ratio Negative) X100

DISTRIBUTION: ENZYME INHIBITION STUDY

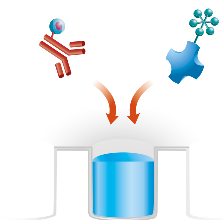
	ENZYMATIC STEP				DETECTION STEP	
	ENZYMATIC BUFFER	INHIBITOR	MLL1/WARD	COFACTOR/ SUBSTRATE MIXTURE	CRYPTATE-Ab	SA-XL 665
SAMPLE	-	4 µL	2 µL	4 µL	5 µL	5 µL
POSITIVE CONTROL	4 µL	-	2 µL	4 µL	5 µL	5 µL
NEGATIVE CONTROL	6 µL	-	-	4 µL	5 µL	5 µL

Enzymatic step



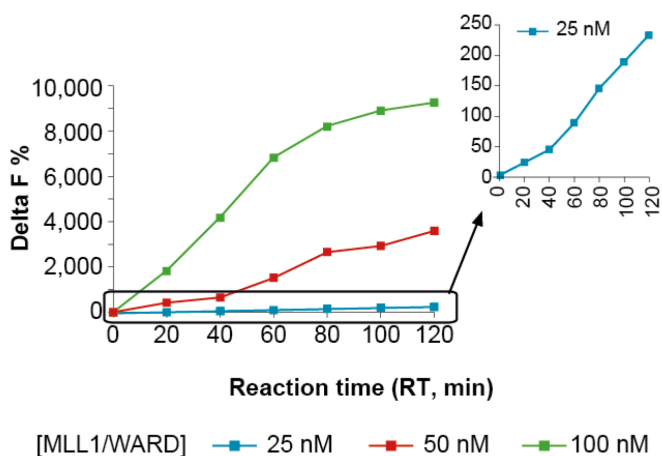
4µL compounds
2µL MLL1/WARD
4µL biotinylated substrate
/cofactors mixture

Detection step



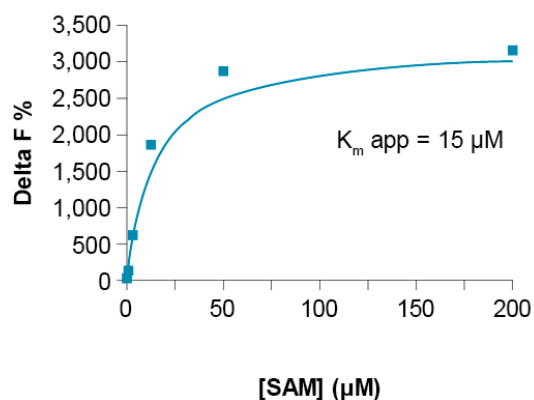
5µL SA-XL665
5µL Anti-Methyl H3 antibody-Cryptate

1. TIME COURSE AND ENZYME TITRATION



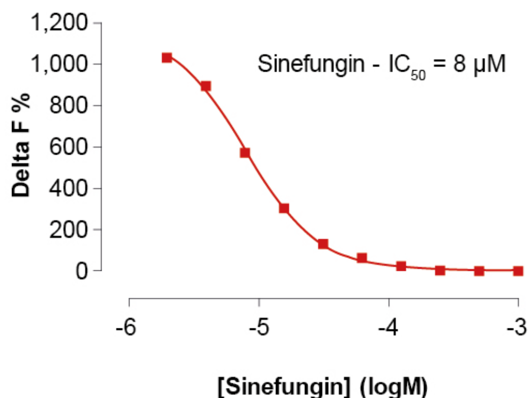
This step allows the optimal enzyme concentration and enzyme reaction time to be determined. Human recombinant MLL1/WARD complex was serially diluted to the concentrations indicated in the figure (25, 50, 100 nM), and the assay was carried out with 200 nM biotinylated H3(1-50) -me0 peptide substrate and 200 µM 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 Km for SAM. The Km 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 µM to 0.195 µM (serial dilutions). The enzyme reaction was stopped at the optimal incubation period (RT, 120 min) by adding the detection reagents. The 15 µM Km 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 µM SAM and 50 nM MLL1/WARD complex. Serial dilutions of sinefungin ranged from 1.9 µM 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 µM SAM. The enzyme reaction was stopped with the detection reagents after 120 min incubation at RT. IC50 value calculated from the inhibition curve was 7.9 µM .

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

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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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