HTRF® SET7/9 HISTONE H3K4 MONO-METHYLATION ASSAY (me0 → me1)

TECHNICAL NOTE

ABSTRACT SET7/9 Histone H3K4 mono-methylation assay measures the monomethylation of a biotinylated histone H3(1-21) peptide at lysine 4.

The HTRF SET7/9 Histone H3K4 monomethylation assay uses a H3(1-21) lysine 4 un-methylated biotinylated peptide (substrate), a Eu3+-cryptate labeled anti-H3K4 me1 detection antibody and XL665-conjugated Streptavidin (SA-XL665).

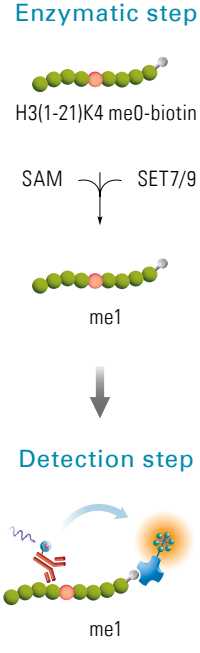
The assay is performed in a single well and run in two steps: the enzymatic step and the detection step. HTRF signal is proportional to the concentration of monomethylated H3(1-21) peptide. The assays within this technical note were performed in a 384-well plate in a 20 µL final volume.

Enzyme	SET7/9
Substrate	H3(1-21)K4 me0-biotin ARTKQTARKSTGGKAPRKQ-LA-GG-K(Biotin)
Detection Antibody	Anti-H3K4 me1-Eu(K)

SET7/9 HISTONE H3K4 MONO-METHYLATION ASSAY AND REAGENTS

H3K4 me1-Eu(K) Ab.	Cisbio Bioassays	# 61KA1KAE
Streptavidin XL-665	Cisbio Bioassays	# 610SAXLA
Detection buffer	Cisbio Bioassays	# 62SDBRDD
SET7/9	BPS Bioscience	# 51010
Histone H3(1-21) lysine 4 un-methylated biotinylated peptide	AnaSpec	# 61702
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.



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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 SET7/9 enzyme (5X)
  - Incubate for 5 min at room temperature
  - 4 µL of H3(1-21)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 me1-Eu(K) 2X according to the product datasheet recommended final concentration and SA-XL665 at 20 nM in detection buffer. Final concentration of 10 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.

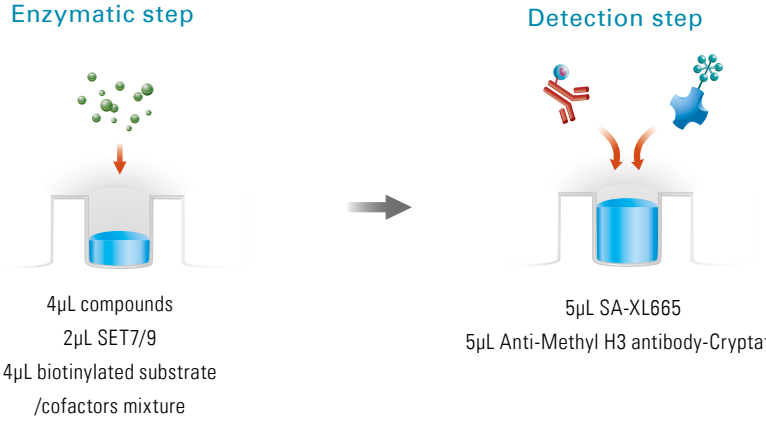
HTRF Ratio = (665nm/620nm)X10^4

Delta Ratio = Sample Ratio - Ratio negative

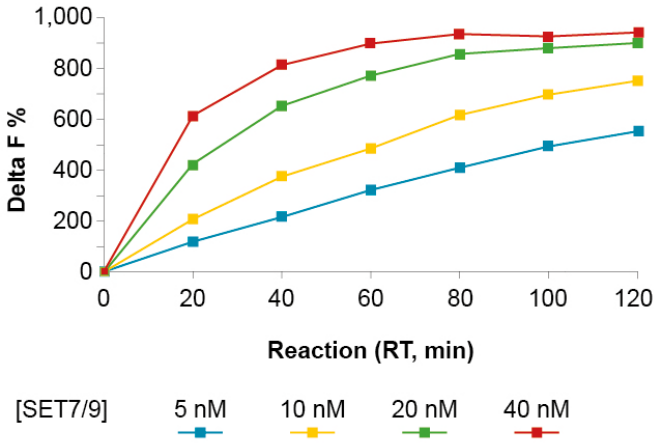
Delta F% = (Delta Ratio/Ratio Negative) X100

DISTRIBUTION: ENZYME INHIBITION STUDY

	ENZYMATIC STEP				DETECTION STEP	
	ENZYMATIC BUFFER	INHIBITOR	SET7/9	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

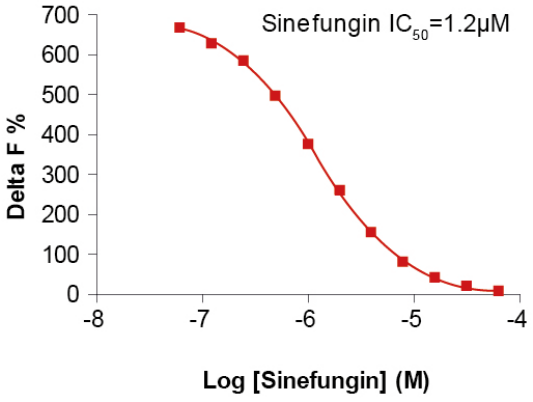


1. TIME COURSE AND ENZYME TITRATION



This step allows the optimal enzyme concentration and enzyme reaction time to be determined. Human recombinant SET7/9 was serially diluted to the concentrations indicated in the figure (5, 10, 20, 40 nM), and the assay was carried out with 40 nM biotinylated H3(1-21)me0 peptide substrate and 200 µM SAM. Enzyme kinetics depends on the SET7/9 specific activity and substrate concentrations. The enzymatic reaction was carried out at RT and then stopped by adding H3K4me1-K Ab and SA-XL665 (detection reagents) after each time point (20, 40, 60, 80, 100, 120 min). A 60 min reaction time using 40 nM SET7/9 was selected for other experiments.

2. ENZYME INHIBITION



SET7/9 H3K4 monomethylation inhibitor assay was validated by measuring activity of the sinefungin inhibitor. This assay was performed using 200 nM SAM and 40 nM SET7/9. Serial dilutions of sinefungin were ranged from 6.1 nM to 100 µM and pre-incubated for 5 min with SET7/9. Enzymatic reaction was initiated by the addition of 80 nM biotinylated H3(1-21) me0 peptide substrate plus 200 nM SAM. The enzyme reaction was stopped with the detection reagents after 60 min incubation at RT. IC50 value calculated from the inhibition curve was 1.2 µM.

For more information, please visit us at www.htrf.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 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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