HTRF G9A Histone H3K27 mono-methylation assay (me0 → me1)

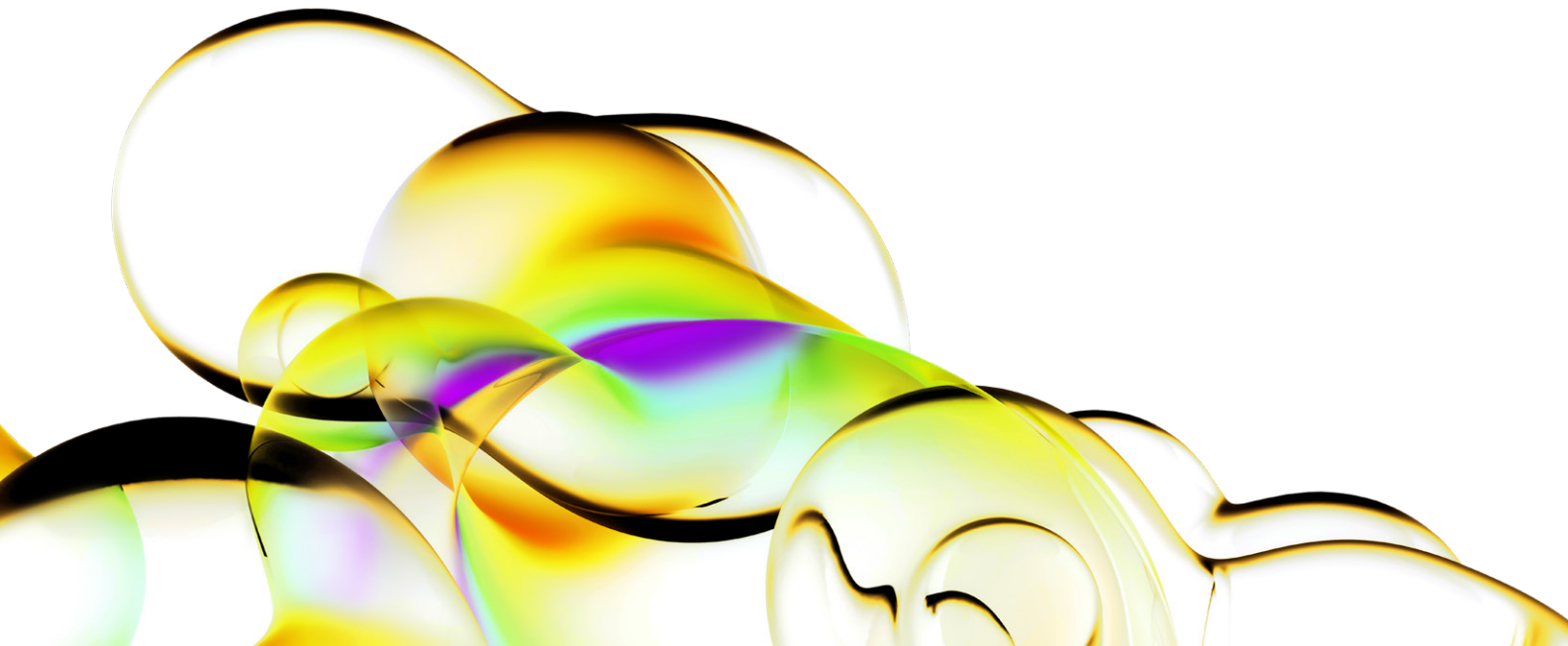
Abstract

G9a Histone H3K27 mono-methylation assay measures the monomethylation of a biotinylated histone H3(21-44) peptide at lysine 27.

The HTRF® G9a Histone H3K27 monomethylation assay uses a H3(21-44) lysine 27 un-methylated biotinylated peptide (substrate), a Eu3+-cryptate labeled anti-H3K27 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(21-44) peptide. The assays within this technical note were performed in a 384-well plate in a 20 µL final volume.

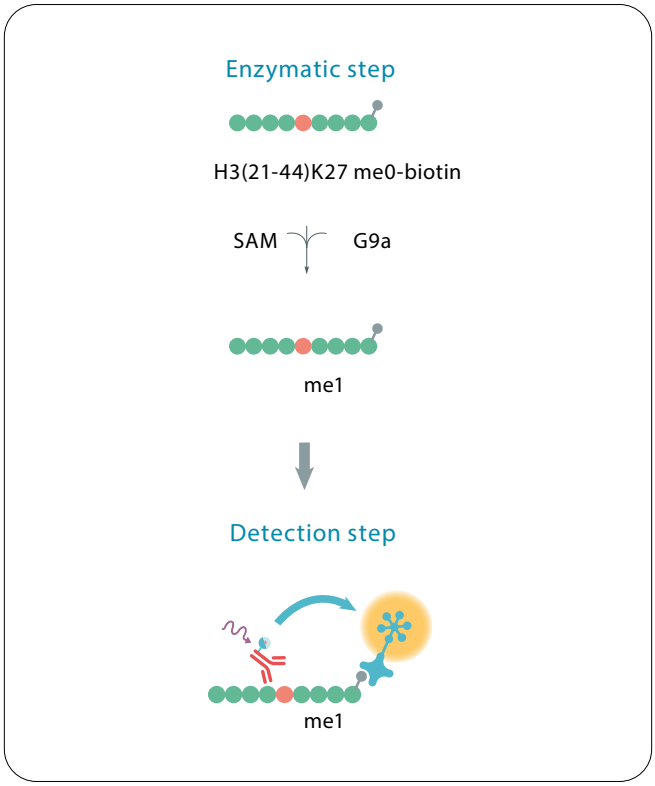
Enzyme	G9a
Substrate	H3(21-44)K27 me0-biotin ATKAARKSAPATGGVKK PHRYRPG-GK(Biotin)
Detection Antibody	Anti-H3K27 me1-Eu(K)



G9A Histone H3K27 mono-methylation assay and reagents

H3K27 me1-Eu(K) Ab.	Revvity	# 61KC1KAE
Streptavidin XL-665	Revvity	# 610SAXLA
Detection buffer	Revvity	# 62SDBRDD
G9a	BPS Bioscience	# 51000
Histone H3(21-44) lysine 27 un-methylated biotinylated peptide	AnaSpec	# 64440
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, 0.01% Tween20	

Data shown on this application note has been obtained using Greiner # 784075, 384-well white microplates.



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 G9a enzyme (5X)
  - Incubate for 5 min at room temperature
  - 4 µL of H3(21-44)K27 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-H3K27 me1-Eu(K) 2X according to the product datasheet recommended final concentration and SA-XL665 at 100 nM in detection buffer. Final concentration of 50 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.

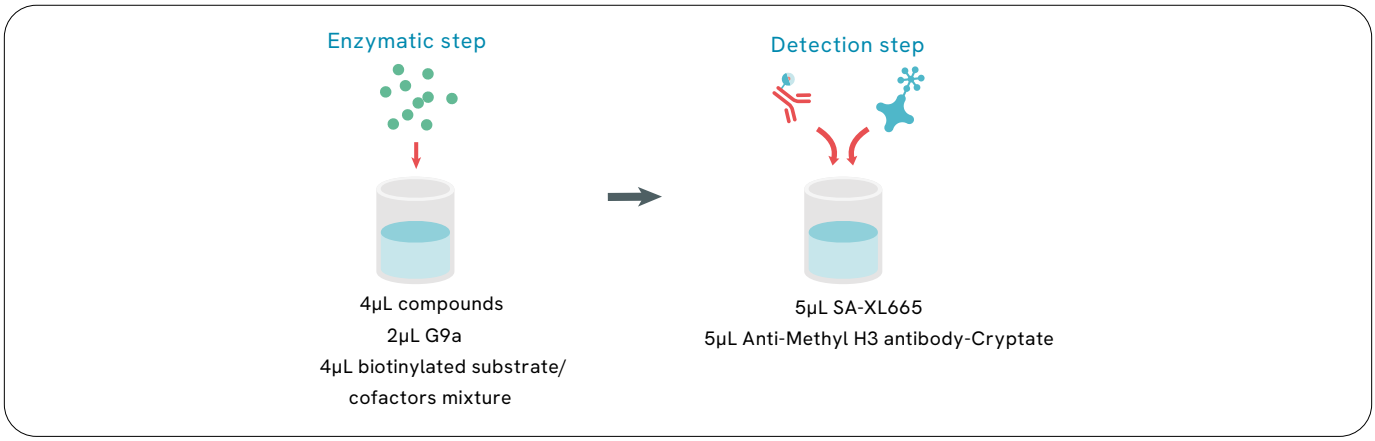
HTRF Ratio = $(665nm/620nm)X10^4$

Delta Ratio = Sample Ratio-Ratio negative

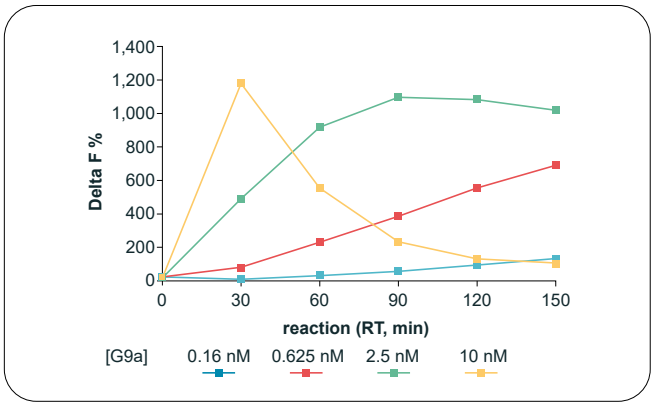
Delta F% = (Delta Ratio/Ratio Negative) X100

Distribution: Enzyme inhibition study

	Enzymatic step				Detection step	
	Enzymatic buffer	Inhibitor	G9A	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

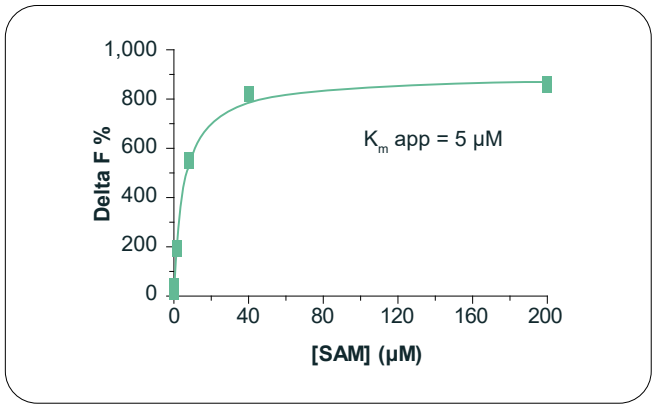


1. Time course and enzyme titration



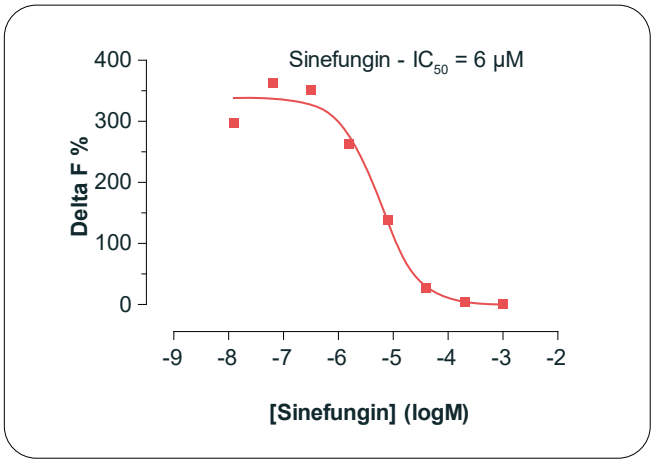
This step allows the optimal enzyme concentration and enzyme reaction time to be determined. Human recombinant G9a was serially diluted to the concentrations indicated in the figure (0.16 ,0.63 ,2.5, 10 nM), and the assay was carried out with 400 nM biotinylated H3(21-44) me0 peptide substrate and 200 µM SAM. Enzyme kinetics depends on the G9a specific activity and substrate concentrations. The enzymatic reaction was carried out at RT and then stopped by adding H3K27me1-K Ab and SA-XL665 (detection reagents) after each time point (30, 60, 90,120,150 min). For further experiments, a reaction time of 180 min at RT and 1.5 nM enzyme were selected.

2. Sam titration



This step enables the determination of Km for SAM. The Km value was determined with 1.5 nM G9a and 400 nM biotinylated H3 (21-44)me0 substrate in the enzymatic step. We recommend testing SAM concentrations ranging from 200 µM to 13 nM (serial dilutions). The enzyme reaction was stopped at the optimal incubation period (RT, 180 min) by adding the detection reagents. The 5 µM Km value for SAM was determined from this experiment using a Michaelis-Menten plot.

3. Enzyme inhibition



G9a H3K27 monomethylation inhibitor assay was validated by measuring activity of sinefungin inhibitor. This assay was performed using 10 µM SAM and 1.5 nM G9a. Serial dilutions of sinefungin were ranged from 13 nM to 1,000 µM and preincubated for 5 min with G9a. Enzymatic reaction was initiated by the addition of 400 nM biotinylated H3 (21-44) me0 peptide substrate plus 10 µM SAM. The enzyme reaction was stopped with the detection reagents after 180 min incubation at RT. IC50 value calculated from inhibition curve was 6 µM.

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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