HTRF® EZH2(Y641F) HISTONE H3K27 TRI-METHYLATION ASSAY (me0 → me3)

TECHNICAL NOTE

ABSTRACT EZH2(Y641F) Histone H3K27 tri-methylation assay measures the trimethylation of a biotinylated histone H3(1-50) peptide at lysine 27.

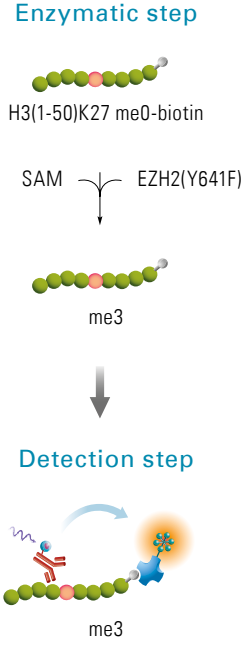
The HTRF EZH2(Y641F) Histone H3K27 trimethylation assay uses a H3(1-50) lysine 27 un-methylated biotinylated peptide (substrate), a Eu3+-cryptate labeled anti-H3K27 me3 detection antibody and XL665-conjugated Streptavidin (SA-XL665).

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	EZH2(Y641F)
Substrate	H3(1-50)K27 me0-biotin ARTKQTARKSTGG-KAPRKQLATKAARKSA-PATGGVKKPHRYRPGTVAL-REGG-K(Biotin)
Detection Antibody	Anti-H3K27 me3-Eu(K)

EZH2(Y641F) HISTONE H3K27 TRI-METHYLATION ASSAY AND REAGENTS

H3K27 me3-Eu(K) Ab.	Cisbio Bioassays	# 61KC3KAE
Streptavidin XL-665	Cisbio Bioassays	# 610SAXLA
Detection buffer	Cisbio Bioassays	# 62SDBRDD
EZH2(Y641F)	BPS Bioscience	# 51017
Histone H3(1-50) lysine 27 un-methylated biotinylated peptide	AnaSpec	# 65366
EZH2 complex	BPS Bioscience	# 51004
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 EZH2(Y641F) enzyme (5X)
  - Incubate for 5 min at room temperature
  - 4 µL of H3(1-50)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 me3-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.

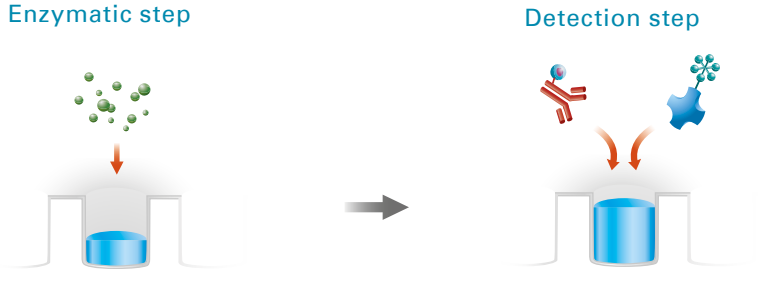
HTRF Ratio = (665nm/620nm)X10^4

Delta Ratio = Sample Ratio - Ratio negative

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

DISTRIBUTION: ENZYME INHIBITION STUDY

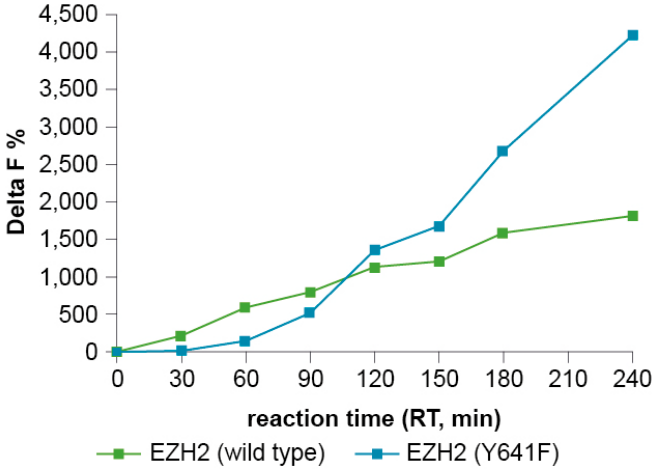
	ENZYMATIC STEP				DETECTION STEP	
	ENZYMATIC BUFFER	INHIBITOR	EZH2(Y641F)	COFACTOR/ SUBSTRATE MIXTURE	CRYPTATE-Ab	SA-XL 665
SAMPLE	-	4 µL	2 µL	4 µL	5 µL	5 µL
POSITIVE CONTROL	4 µL	-	2 µL	4 µL	5 µL	5 µL
NEGATIVE CONTROL	6 µL	-	-	4 µL	5 µL	5 µL



4µL compounds
2µL EZH2(Y641F)
4µL biotinylated substrate
/cofactors mixture

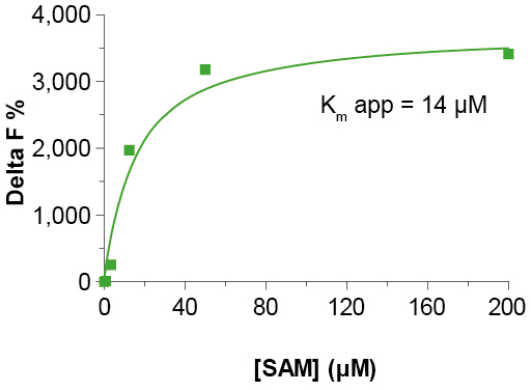
5µL SA-XL665
5µL Anti-Methyl H3 antibody-Cryptate

1. TIME COURSE



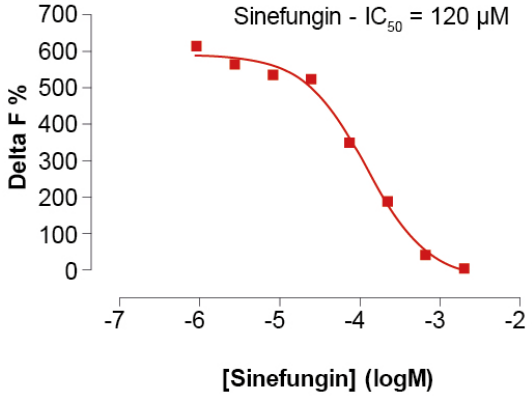
In this experiment, time course reaction of two types of human recombinant EZH2 complex (wild type and Y641 mutant) were compared at 30 °C . Both complex enzymes were added at 50 ng/well. This assay was carried out with 400 nM biotinylated H3(1-50)me0 peptide substrate and 200 µM SAM and the reaction was then stopped by adding H3K27me3-K Ab and SA-XL665 (detection reagents) after each time poin t(30, 60, 90, 120, 150, 180, 210, 240 min). For further experiments, a reaction time of 180 min at RT and 40 ng/well enzyme complex were selected.

2. SAM TITRATION



This step enables the determination of Km for SAM. The Km value was determined with 40 ng/well EZH2(Y641) complex and 400 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, 180 min) by adding the detection reagents. The 14 µM Km value for SAM was determined from this experiment using a Michaelis-Menten plot.

3. ENZYME INHIBITION



EZH2 H3K27 trimethylation inhibitor assay was validated by measuring the activity of sinefungin inhibitor. This assay was performed using 15 µM SAM and 40 ng/well EZH2(Y641) complex. Serial dilutions of sinefungin ranged from 1 µM to 2 mM and were pre-incubated for 5 min with EZH2(Y641) complex. Enzymatic reaction was initiated by the addition of 400 nM biotinylated H3 (1-50) peptide substrate plus 15 µM SAM. The enzyme reaction was stopped with the detection reagents after 180 min incubation at RT. IC50 value calculated from the inhibition curve was 120 µM.

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

RELATED ARTICLES

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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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