

HTRF LSD1 histone H3K4 mono-demethylation assay (me1 → me0).

Abstract

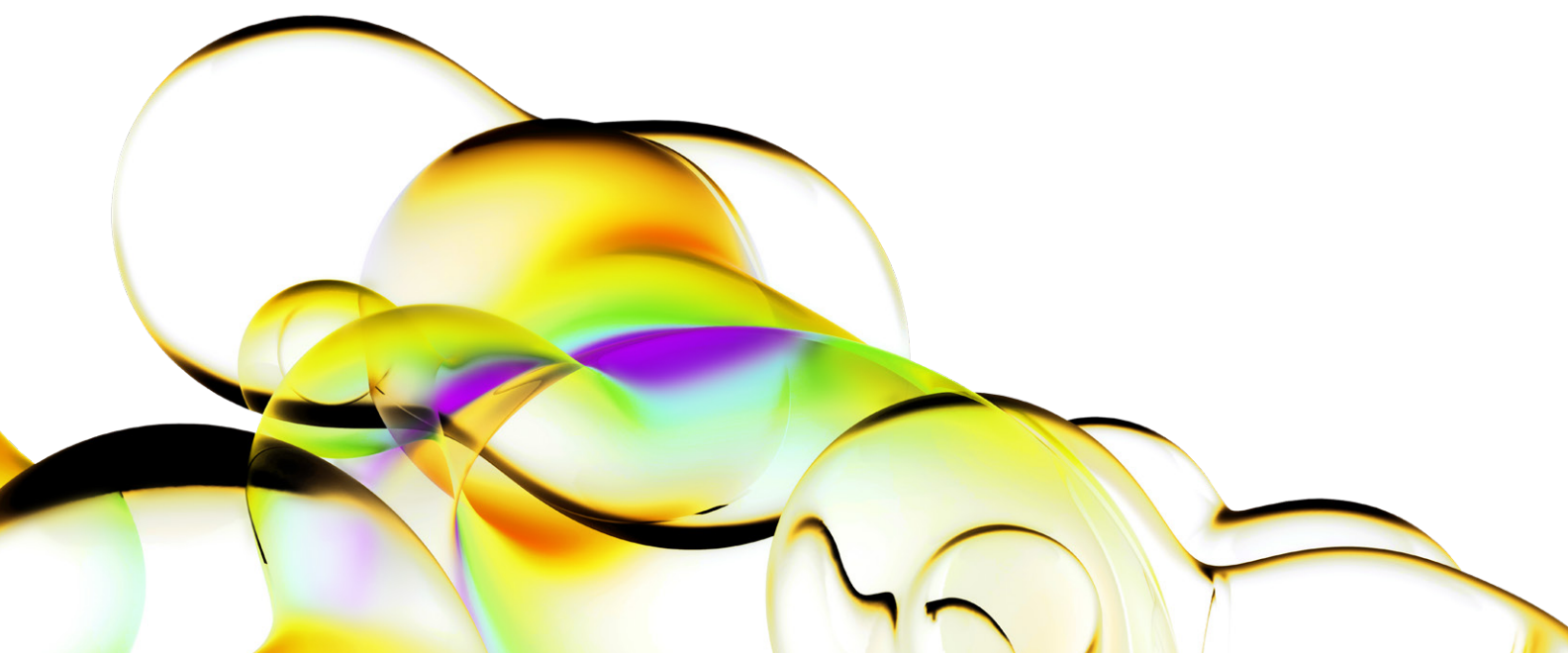
LSD1 Histone H3K4 mono-demethylation assay measures the demethylation of a biotinylated histone H3(1-21) peptide at lysine 4.

The HTRF® LSD1 Histone H3K4 demethylation assay uses a H3(1-21) lysine 4 mono-methylated biotinylated peptide (substrate), a Eu3+-cryptate labeled anti-H3K4 me0 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 demethylated H3(1-21) peptide. The assays within this technical note were performed in a 384-well plate in a 20 µL final volume.

Enzyme	LSD1
Substrate	H3(1-21)K4 me1-biotin ART-K(Me1)-QTARKSTGG- KAPRKQLA- GGK(Biotin)
Detection Antibody	Anti-H3K4 me0-Eu(K)

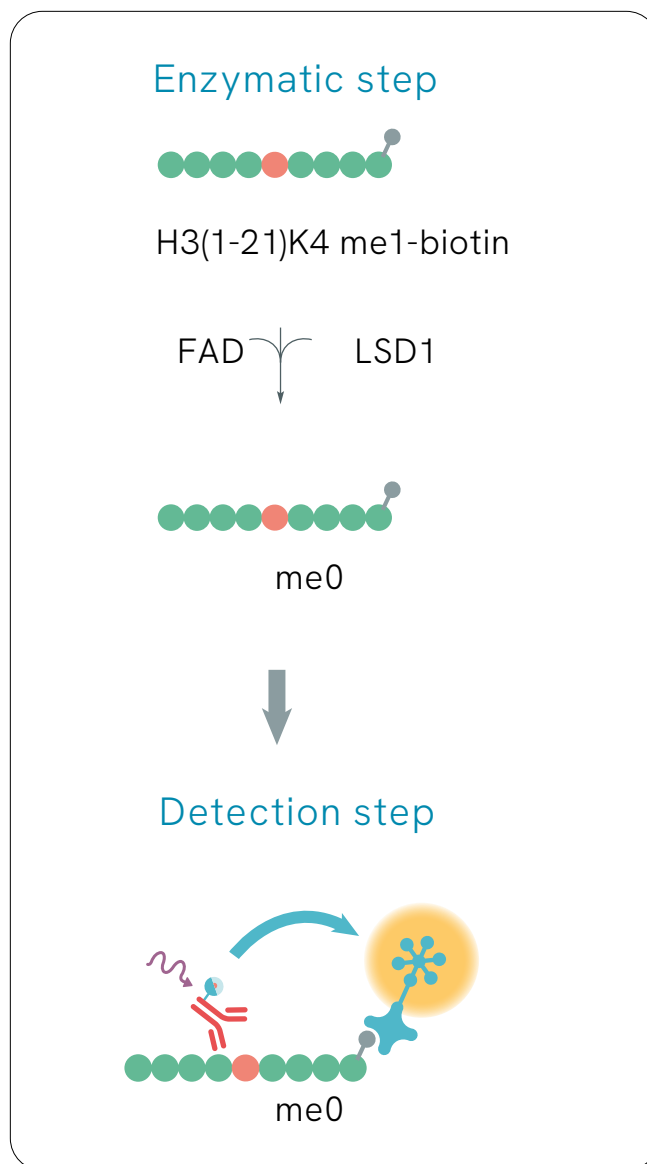
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LSD1 histone H3K4 mono-demethylation assay and reagents

H3K4 me0-Eu(K) Ab.	Revvity	# 61KA0KAE
Streptavidin XL-665	Revvity	# 610SAXLA
Detection buffer	250 mM NaKPO4 pH 6.0, 0.1% BSA, 0.8M KF	
LSD1	BPS Bioscience	# 50100
Histone H3(1-21) lysine 4 mono-methylated biotinylated peptide	AnaSpec	# 64355
CoREST	BPS Bioscience	# 50274
FAD (flavin adenine dinucleotide disodium salt hydrate)	Sigma	# F6625
Tranlycypromine hydrochloride	Stemgent	#04-0033
Enzymatic buffer	50 mM Tris-HCl pH 8.5, 50 mM NaCl, 1 mM DTT, 10 µM FAD, 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>.



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 LSD1 enzyme (5X)
 - Incubate for 5 min at room temperature
 - 4 µL of H3(1-21)K4 me1-biotin peptide/FAD 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 me0-Eu(K) 2X according to the product datasheet recommended final concentration and SA-XL665 at 10 nM in detection buffer. Final concentration of 5 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 665 nM and 620 nM wavelengths on an HTRF compatible reader.

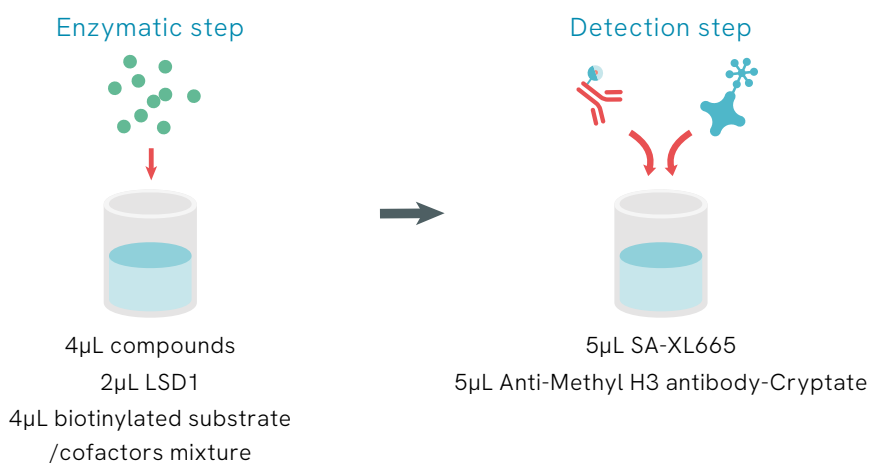
$$\text{HTRF Ratio} = (665 \text{ nM}/620 \text{ nM}) \times 10^4$$

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

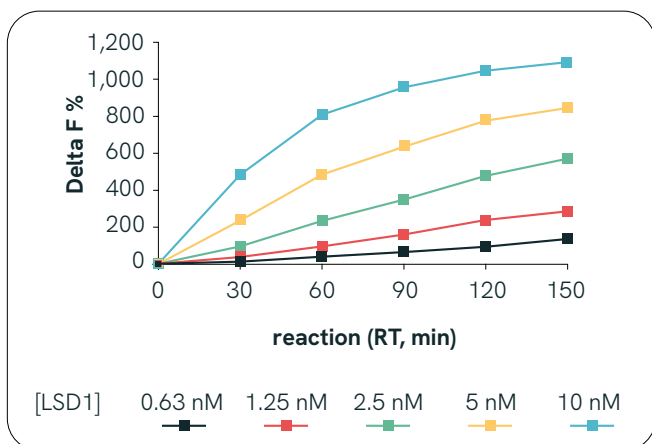
$$\text{Delta F\%} = (\text{Delta Ratio}/\text{Ratio Negative}) \times 100$$

Distribution: Enzyme inhibition study

	Enzymatic step				Detection step	
	Enzymatic buffer	Inhibitor	LSD1	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

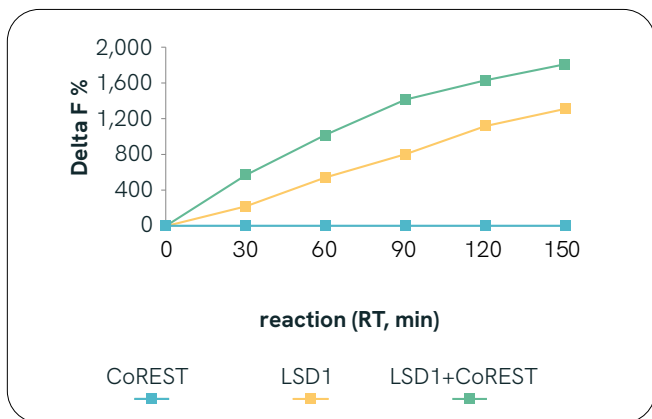


1. Time course and enzyme titration



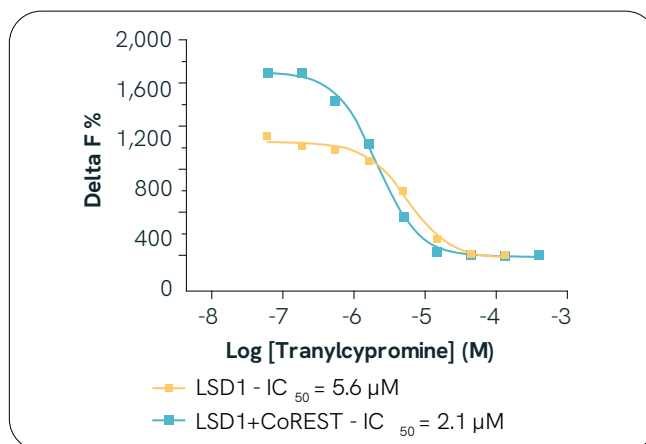
This step allows the optimal enzyme concentration and enzyme reaction time to be determined. Human recombinant LSD1/CoREST complex was serially diluted to the concentrations indicated in the figure (LSD1; 0.63, 1.25, 2.5, 5, 10 nM), and the assay was carried out with 10 nM biotinylated H3K4(1-21) me1 peptide substrate and 10 μ M FAD. Enzyme kinetics depends on the LSD1 specific activity and substrate concentrations. The enzymatic reaction was carried out at RT and then stopped by adding H3K4me0-K Ab and SA-XL665 (detection reagents) after each time point (30, 60, 90, 120, 150 min). A 60 min reaction time using 5 nM LSD1 was selected for other experiments.

2. LSD1 and corest complex



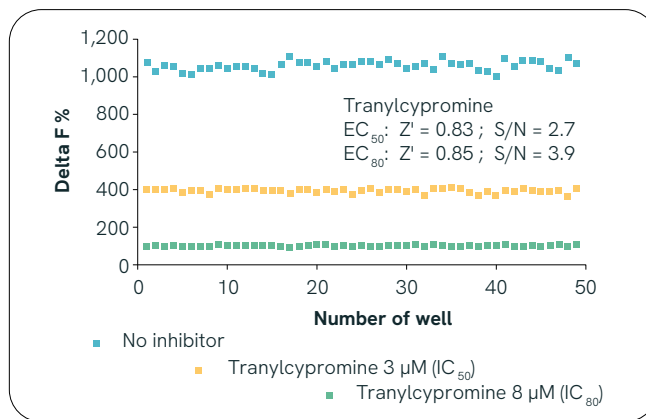
This step showed the additive effect of LSD1 and CoREST for H3K4 demethylation. The enzyme reaction was carried out with 5 nM LSD1, 10 nM CoREST and 20 nM H3K4(1-21) me1 substrate at RT. CoREST enhanced H3K4 demethylation effect from LSD1. For further experiments, 40 nM peptide substrate, a reaction time of 60 min at RT and 5 nM LSD1 and 10 nM CoREST were selected.

3. Enzyme inhibition



LSD1 H3K4 mono-demethylation inhibitor assay was validated by measuring the activity of tranylcypromine inhibitor. This assay was performed using 5 nM LSD1 and 10 nM CoREST. Serial dilutions of tranylcypromine ranged from 0.18 μ M to 400 μ M and were pre-incubated for 5 min with LSD1 enzyme. Enzymatic reaction was initiated by the addition of 40 nM biotinylated H3K4(1-21) me1 peptide substrate. The enzyme reaction was stopped with the detection conjugates after 60 min incubation at RT. IC₅₀ values were calculated from inhibition curves of LSD1 only and LSD1 and CoREST complex. They are respectively 5.6 and 2.1 μ M.

4. Z' factor determination



The robustness of the assay was proven by performing a Z' determination with tranylcypromine at IC₅₀ (3 μ M) and IC₈₀ (8 μ M). The enzyme reaction was carried out with 5 nM LSD1, 10 nM CoREST and 40 nM H3K4(1-21)me1 substrate for 60 min at RT. Z' of 0.83 and 0.85 show the robustness of the assay.

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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