HTRF® JARID1A HISTONE H3K4 MONO-DEMETHYLATION ASSAY (me3 → me2)

TECHNICAL NOTE

ABSTRACT JARID1A Histone H3K4 mono-demethylation assay measures the demethylation of a biotinylated histone H3(1-21) peptide at lysine 4.

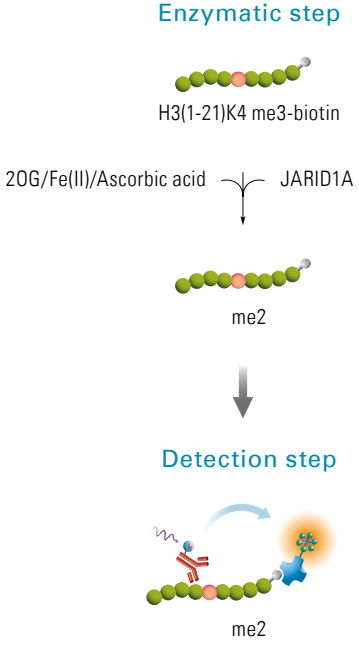
The HTRF JARID1A Histone H3K4 demethylation assay uses a H3(1-21) lysine 4 tri-methylated biotinylated peptide (substrate), a Eu3+-cryptate labeled anti-H3K4 me2 detection antibody and XL665-conjugated Streptavidin (SA-XL665).

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	JARID1A
Substrate	H3(1-21)K4 me3-biotin ART-K(Me3)-QTARKSTGG-KAPRKQLA-GGK(Biotin)
Detection Antibody	Anti-H3K4 me2-Eu(K)

JARID1A HISTONE H3K4 MONO-DEMETHYLATION ASSAY AND REAGENTS

H3K4 me2-Eu(K) Ab.	Cisbio Bioassays	# 61KA2KAE
Streptavidin XL-665	Cisbio Bioassays	# 610SAXLA
Detection buffer	Cisbio Bioassays	# 62SDBRDD
JARID1A	BPS Bioscience	# 50110
Histone H3(1-21) lysine 4 tri-methylated biotinylated peptide	AnaSpec	# 64357
Ammonium iron(II) sulfate hexahydrate (Fe(II))	Sigma	# F3754
L-ascorbic acid (Asc)	Sigma	# A5960
alpha-ketoglutaric acid disodium salt (2OG)	Sigma	# K3752
N-Oxalylglycine	Sigma	# O9390
Enzymatic buffer	50 mM MES pH6.5, 50 mM NaCl, 0.01% tween20 plus 2mM ascorbic acid	



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.

cisbio
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 JARID1A enzyme (5X)
  - Incubate for 5 min at room temperature
  - 4 µL of H3(1-21)K4 me3-biotin peptide/ 2OG/Fe(II) 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 me2-Eu(K) 2X according to the product datasheet recommended final concentration and SA-XL665 at 25 nM in detection buffer. Final concentration of 12.5 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 665nm and 620nm wavelengths on an HTRF compatible reader.

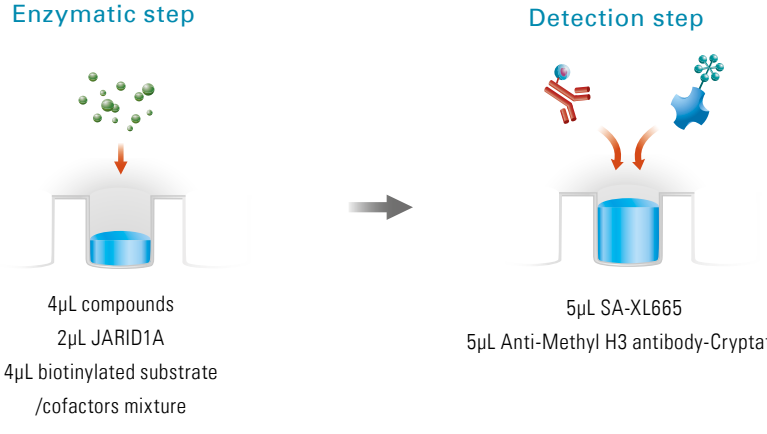
HTRF Ratio = (665nm/620nm)X10^4

Delta Ratio = Sample Ratio - Ratio negative

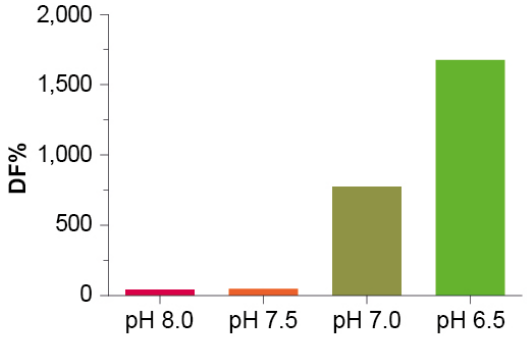
Delta F% = (Delta Ratio/Ratio Negative) X100

DISTRIBUTION: ENZYME INHIBITION STUDY

	ENZYMATIC STEP				DETECTION STEP	
	ENZYMATIC BUFFER	INHIBITOR	JARID1A	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



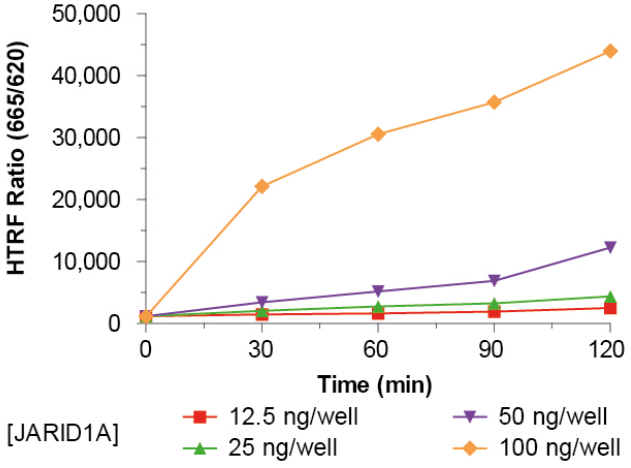
1. ENZYMATIC BUFFER OPTIMIZATION



pH 8.0 : 50 mM Tris pH8, 50 mM NaCl, 0.01% tween20
pH 7.5 : 50 mM Tris pH7.5, 50 mM NaCl, 0.01% tween20
pH 7.0 : 50 mM HEPES pH7, 50 mM NaCl, 0.01% tween20
pH 6.5 : 50 mM MES pH6.5, 50 mM NaCl, 0.01% tween20

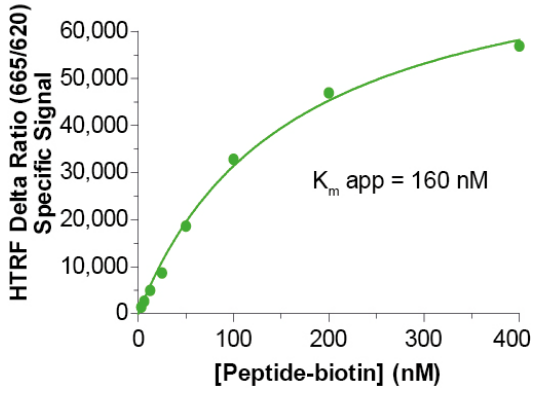
This step allows the optimal enzymatic buffer pH to be determined. The assay was carried out with 50ng/well JARID1A, 100 nM biotinylated H3K4(1-21)me3 peptide substrate, 1 mM 2-OG and 50µM Fe(II). The enzymatic reaction was carried out at RT for 90min and then stopped by adding H3K4me2-K Ab and SA-XL665 (detection reagents). A neagtive control without enzyme was performed in order to determined the non specific signal and calculate the DF%. For further experiments 50 mM MES pH6.5, 50 mM NaCl, 0.01% tween20 buffer was selected.

2. TIME COURSE AND ENZYME TITRATION



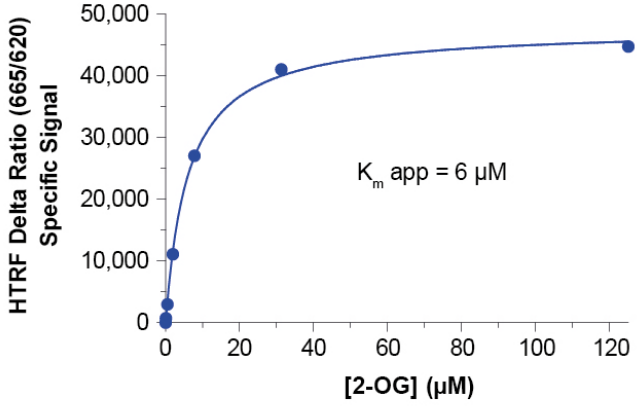
This step allows the optimal enzyme concentration and enzyme reaction time to be determined. Human recombinant JARID1A was serially diluted to the concentrations indicated in the figure (12.5, 25, 50, 100 ng/well), and the assay was carried out with 100 nM biotinylated H3K4(1-21) me3 peptide substrate, 1 mM 2-OG and 50µM Fe(II). Enzyme kinetics depends on the JARID1A specific activity and substrate concentrations. The enzymatic reaction was carried out at RT and then stopped by adding H3K4me2-K Ab and SA-XL665 (detection reagents) after each time point (0, 30, 60, 90, 120 min). For further experiments a reaction time of 90min at RT, and 70 ng/well enzyme were selected.

3. PEPTIDE TITRATION



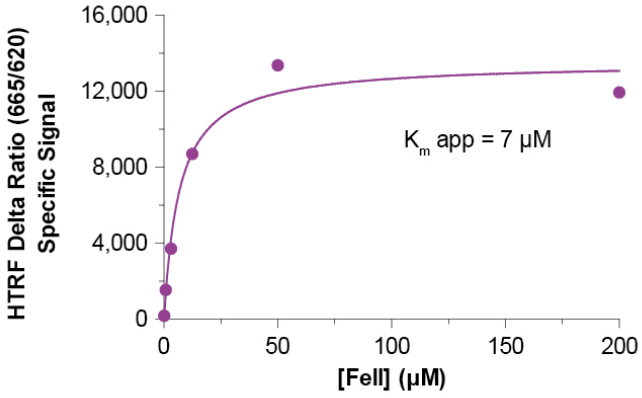
This step allows the determination of apparent Km for peptide. The Km value was determined with 70 ng/well JARID1A, 50µM Fe(II) and 1 mM 2-OG in the enzymatic step. We recommend testing biotinylated H3K4(1-21)me3 substrate concentrations ranging from 500 nM to 1 nM (serial dilutions). The streptavidin XL-665 concentration varie according to the peptide concentration keeping constant the ratio of 1/4 (Streptavidin XL-665 / peptide). For each concentration of peptide and streptavidin XL-665, a negative control is performed by removing the 2-OG and peptide from the wells. This negative control is used as non specific signal to calculate the HTRF delta ratio (hence specific signal). The enzyme reaction was stopped by adding the detection reagents at the optimal incubation period (RT, 120 min). The 160 nM apparent Km value for peptide was determined from this experiment using a Michaelis-Menten plot.

4. 2-OG TITRATION



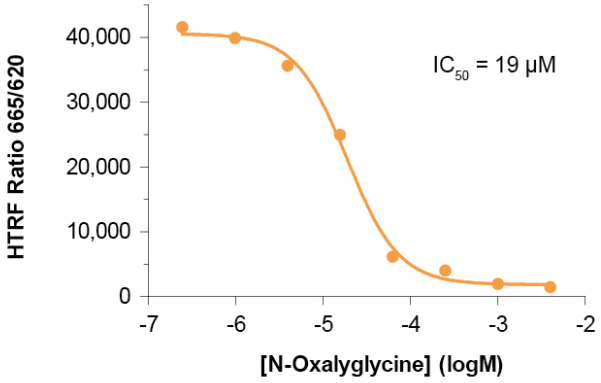
This step allows the determination of apparent Km for 2-OG. The Km value was determined with 70 ng/well JARID1A, 100 nM biotinylated H3K4(1-21)me3 substrate and 50 µM Fe(II) in the enzymatic step. We recommend testing 2-OG concentrations ranging from 150 µM to 0.1 µM (serial dilutions). A negative control is performed by removing the 2-OG and peptide from the wells. This negative control is used as non specific signal to calculate the HTRF delta ratio (hence specific signal). The enzyme reaction was stopped by adding the detection reagents at the optimal incubation period (RT, 120 min). The 6 µM apparent Km value for 2-OG was determined from this experiment using a Michaelis-Menten plot.

5. FE(II) TITRATION



This step allows the determination of apparent Km for Fe(II). The Km value was determined with 70ng/well JARID1A, 100 nM biotinylated H3K4(1-21)me3 substrate and 1 mM 2-OG in the enzymatic step. We recommend testing Fe(II) concentrations ranging from 100 µM to 0.1 µM (serial dilutions). A negative control is performed by removing the Fe(II) and peptide from the wells. This negative control is used as non specific signal to calculate the HTRF delta ratio (hence specific signal). The enzyme reaction was stopped by adding the detection reagents at the optimal incubation period (RT, 120 min). The 7 µM Km value for Fe(II) was determined from this experiment using a Michaelis-Menten plot.

6. ENZYME INHIBITION



JARID1A H3K4 demethylation inhibitor assay was validated by measuring activity of known inhibitor. This assay was performed using 10µM 2-OG, 50 µM Fe(II) and 70 ng/well JARID1A. Serial dilutions of inhibitors were pre-incubated for 5 min with JARID1A. Enzymatic reaction was initiated by the addition of 100 nM biotinylated H3 (1-21) K4me3 peptide substrate plus 10 µM 2-OG. The enzyme reaction was stopped with the detection reagents after 120 min incubation at RT. IC50 values were calculated from inhibition curves.

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.

FOR MORE INFORMATION
Europe and other countries +33(0)466 796 705 U.S. and Canada 1-888-963-4567 China +86 10 8080 9288
Visit www.htrf.com to find a list of our regional distributors

cisbio
INTERACTION IS EVERYTHING