Datasheet for ABIN577652

Histone Demethylase Fluorescent Kit

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Overview

Quantity:	2 x 96 tests
Target:	HDM
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The DetectX® Demethylase Activity kit is designed to quantitatively measure the enzymatic activityof formaldehyde-producing enzymes such as Histone Demethylases.
Brand:	DetectX®
Sample Type:	Cell Lysate
Detection Method:	Fluorometric
Specificity:	Sample Types validated: LSD1-and Jumonji-type Demethylases
Characteristics:	The Universal Histone Demethylase (HDM) Fluorescent Activity kit allows all known HDMs to be measured in low, medium and high throughput fashion. Run the demethylase reaction and after completion simply add the supplied Formaldehyde Detection Reagent to each well and read the fluorescent signal generated. Excitation at 450nm/Emission at 510nm using an adjustable gain plate fluorimeter. Formaldehyde is a common byproduct formed in oxidative demethylation. Examples of formaldehyde-producing enzymes include histone demethylases (HDMs) that modify methylated histones. Lysine-specific HDMs were first discovered in 2004 and are currently among the most actively studied formaldehyde-producing enzymes. HDMs catalyze the site-specific demethylation of methyl-lysine residues in histones to dynamically regulate chromatin structure, gene expression, and potentially other genomic functions. At present, there are two known classes of HDMs: the flavin adenine nucleotide (FAD)-dependent Lysine

Product Details

	Specific Demethylase 1 (LSD1) family and the Fe(II)-dependent Jumonji C (JmjC) family. Although the LSD1 and JmjC HDMs employ different cofactors and catalytic mechanisms, both produce formaldehyde as a byproduct of the demethylation reaction. Despite their biological importance, HDMs have proven difficult to quantitatively assay owing to their relatively low turnover numbers, hindering our understanding of their kinetic properties, substrate specificities, and reaction mechanisms.
Components:	Black Half Area 96 Well Plate 2 plates LSD1-type Assay Buffer 60 mL A phosphate buffer containing detergents and stabilizers. JMJD2A-type Assay Buffer 60 mL A HEPES buffer containing stabilizers. Formaldehyde Standard 0.5 mL 2,000 µM formaldehyde solution in a special stabilizing solution. Outer container has formalde- hyde absorbing pad. The standard is stable if kept tightly sealed. KEEP TIGHTLY SEALED DetectX® Formaldehyde Reagent 5 mL Special formulation of reagents to detect formaldehyde in solution. Contains ≤0.09% sodium azide as a preservative. Demethylase Cell Lysis Buffer (CLB) 100 mL A Tris based buffer containing detergents. Store Frozen as this buffer contains no preservatives. Plate Sealers 2 Each
Material not included:	Supply of distilled or deionized water free of formaldehyde. Repeater pipet with disposable tips capable of dispensing 25 µL. Incubators capable of accurately maintaining 30 °C and 37 °C. Demethylase enzyme samples. A source of LSD1-type or Jumonji-type demethylase, along with any cofactors, enzyme substrates, inhibitors, and/or activators. Fluorescence 96 well plate reader capable of reading fluorescent emission at 510 nm, with excita- tion at 450 nm. Set plate parameters for a 96-well Corning Costar 3694 plate. See: http://www. ArborAssays.com/resources/lit.asp for plate dimension data. Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

Target Details

Target:	HDM
Background:	Formaldehyde is a common by-product formed in the oxidative demethylation of proteins,

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nucle- ic acids, and biological small molecules. Examples of formaldehyde-producing enzymes include DNA demethylases, histone demethylases (HDMs), and cytochrome P450 enzymes that demethy- late drugs and other xenobiotic compounds1-6. HDMs catalyze the site-specific demethylation of methyl-lysine residues in histones to dynamically regulate chromatin structure, gene expression, and potentially other genomic functions. Lysine-specific HDMs were first discovered in 2004 and are currently among the most actively studied formaldehyde-producing enzymes7. At present, there are two known classes of HDMs: the flavin adenine dinucleotide (FAD)-dependent Lysine Specific Demethylase 1 (LSD1) family and the Fe(II)-dependent Jumonji C (JmjC) family. Although the LSD1 and JmjC HDMs employ different cofactors and catalytic mechanisms (see below), both produce formaldehyde as a byproduct of the demethylation reaction. Despite their biological importance, HDMs have proven difficult to quantitatively assay owing to their relatively low turnover numbers, hindering our understanding of their kinetic properties, substrate specificities, and reaction mecha- nisms

Application Details

Application Notes:	Histone demethylases diluted in the assay buffers provided are compatible with this assay. For HDM samples in cell lysates, we include a specially formulated Cell Lysis Buffer, X050-100ML, that has been shown not to interfere with formaldehyde detection. Cell lysis buffers containing SDS and Triton X-100 inhibit the formaldehyde signal reaction and should not be used. NOTE: Cell lysates made in CLB can be measured in the assay directly. The standards for the formaldehyde standard curve should be made in CLB.
Protocol:	The kit is unique in that the product of these enzymatic demethylation reactions, formaldehyde, is quantitated directly by a fluorescent product. No separation or washing is required. The kit has been validated for both LSD1 and JMJD2A histone Demethylases (HDMs). The kit provides optimized buffers for the HDMs, LSD1 and JMJD2A, a stable formaldehyde stan- dard, the Formaldehyde Detection Reagent (FDR) and two 96 well plates for detecting the gen- erated fluorescent signal. The kit allows any enzymatic reaction generating formaldehyde to be measured. The end user will have to provide the demethylase system and any cofactors, etc. necessary for activity, along with any test inhibitors or activators. The kit allows end users to pro- duce HDM activity in many in vivo and in vitro systems and then determine the activity by measuring formaldehyde generation. For in vitro studies, the HDM reaction should be carried out in our supplied buffers using

	optimized reaction conditions for the demethylation. For HDM samples in cell lysates, we include a specially formulated Cell Lysis Buffer, X050-100ML, that has been shown not to interfere with formaldehyde detection. Cell lysis buffers containing SDS and Triton X-100 inhibit the formaldehyde signal reac- tion and should not be used. Following the formaldehyde generating reaction, the reaction can be stopped by addition of a suitable inhibitor. The FDR is then added to all the wells. If calibration to formaldehyde is needed (for cross lab comparisons) then a formaldehyde standard curve generated from the supplied standard should be run. After a short incubation at 37 °C for 30 minutes, the fluorescent product is read at 510 nm in a fluorescent plate reader with excitation at 450 nm. The demethylase activity is determined based upon formaldehyde production, after making a suitable correction for any dilution of the sample, using software available with most fluorescence plate readers. We have provided two 96 well plates for measurement but this assay is adaptable for higher density plate formats. The end user should ensure that their HTS black plate is suitable for use with these reagents prior to running samples.
Reagent Preparation:	Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine activity. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. Standard Preparation Label seven glass test tubes as #1 through #7. Pipet 450 µL of Assay Buffer containing all cofac- tors and additives into tube #1 and 250 µL into tubes #2-#7. Add 50 µL of the Formaldehyde stock solution to tube #1 and vortex completely. Add 250 µL of tube #1 to tube #2 and vortex com- pletely. Repeat these serial dilutions for tubes #3 through #7. The concentration of formaldehyde in tubes 1 through 7 will be 200, 100, 50, 25, 12.5, 6.25 and 3.125 µM. Use all Standards within 1 hour of preparation Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Std 7 Assay Buffer Volume (µL) 450 250 250 250 250 250 250 Addition Stock Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Volume of Addition (µL) 50 250 250 250 250 250 250 Final Conc (µM) 200 100 50 25 12.5

	6.25 3.125 NOTE: Cell lysates made in CLB can be measured in the assay directly. The standards for the formaldehyde standard curve should be made in CLB.
Assay Procedure:	Demethylase reaction volume should be no more than 100 µL in each well including all cofactors, inhibitors and activators diluted into the kit Assay Buffer or CLB for cell lysates. Demethylase Reaction 1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. 2. Set up the appropriate demethylase reaction in one of the supplied buffers. 3. Pipet 100 µL standards or samples plus all cofactors and inhibitors into duplicate wells in the black plate. 4. Pipet 100 µL Assay Buffer or CLB plus all cofactors and inhibitors into duplicate wells as a Zero standard. 5. Carry out demethylation reaction and preferably stop reaction at an appropriate time. Formaldehyde Detection 6. Add 25 µL of the DetectX® Formaldehyde Detection Reagent to each well using a repeater pipet. 7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. 8. Incubate at 37 °C for 30 minutes. Room temperature incubation will yield approximately 75 % of the fluorescent signal generated with 37 °C incubation. 9. Set plate parameters for a 96-well Corning Costar 3694 plate. See: http://www.ArborAssays.com/resources/lit.asp for plate dimension data. Read the fluorescent signal from each well in a plate reader capable of reading the fluorescent signal at 510 nm with excitation at 450 nm. Please contact your plate reader manufacturer for suitable filter sets. 10. Use the plate reader's built-in 4PLC software capabilities to calculate formaldehyde concentrations for each sample.
Calculation of Results:	Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero standard. The sample activity obtained should be multiplied by the dilution fac- tor to obtain neat sample values. Or use the online tool from http://www.myassays.com/arbor-assays-histone-demethylase-fluores- cent-activity-kit.assay to calculate the data. * *The MyAssays logo is a registered trademark of MyAssays Ltd. tyPical data - lsd1 assay LSD1 Conc. (µM) Mean FLU Net FLU Zero 1,827 0 0.64 18,522 16,695 0.256 12,410 10,584 0.128 6,849 5,022 tyPical data - JmJd2a assay JMJD2A Conc. (µM) Mean FLU Net FLU Zero 3,028 0 10 17,973 14,945 5 10,719 7,691 2.5

Application Details

	4,528 1,500 Always run your own standard curve for calculation of results. Do not use this data.
Restrictions:	For Research Use only

Handling

Precaution of Use:	As with all such products, this kit should only be used by qualified personnel who have had labo- ratory safety instruction. The complete insert should be read and understood before attempting to use the product. Formaldehyde is a toxic, volatile, reactive chemical that can form adducts with proteins and nucle- ic acids. It reacts with oxygen to form formic acid and so should be kept sealed and only used in well-ventilated laboratories. For disposal, we suggest discarding all excess standards and samples in a 10% aqueous solution of sodium bisulfite, such as Sigma catalog number 13438. Some of the components of this kit contain sodium azide as a preservative, which may react with lead or copper plumbing to form potentially explosive complexes. When disposing of reagents al- ways flush with large volumes of water to prevent azide build-up.
Storage:	-20 °C,4 °C
Storage Comment:	All components of this kit should be stored at 4°C, except the CLB which should be stored at -20°C, until the expiration date of the kit.

Publications

Product cited in:	Alahari, Post, Rolfo, Weksberg, Caniggia: "Compromised JMJD6 histone demethylase activity impacts on VHL gene repression in preeclampsia." in: **The Journal of clinical endocrinology and metabolism**, (2018) (PubMed).

Images

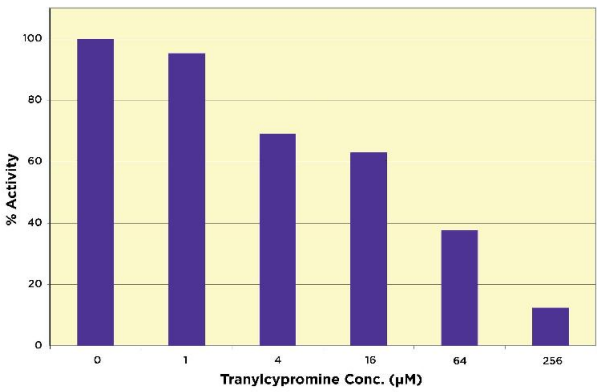

Image 1. LSD Inhibition Data

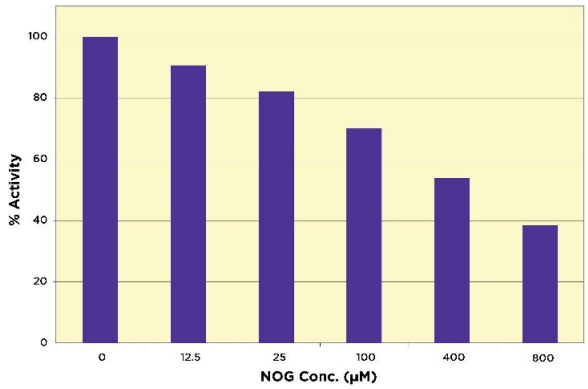

Image 2. NOG Inhibition Data