Product Information

Histone Deacetylase 3 (HDAC3) Activity Assay Kit

EPI004

Storage Temperature –20 °C

Product Description

Histone deacetylases (HDACs) are a large family of enzymes that remove acetyl groups from histone proteins. Site specific histone acetylation and deacetylation have been shown to activate or repress eukaryotic gene transcription, respectively, and as a consequence, it plays a crucial role in mammalian development and disease. HDACs are involved in important biological activities, such as cell differentiation, proliferation, apoptosis, and senescence.

With Sigma's HDAC3 Activity Assay Kit, HDAC3 present in a test sample will act with the supplied Developer, to deacetylate and then cleave the HDAC3 Substrate [R-H-K-K(Ac)-AFC]. This activity will release the quenched fluorescent group, AFC, which can be detected at $\lambda_{Ex}/\lambda_{Em}$ = 380/500 nm. Trichostatin A is an HDAC inhibitor included in the kit to verify HDAC3 activity. The kit provides a rapid, simple, sensitive and reliable test. It is suitable for either individual tests, or high throughput assays, from nuclear extracts, purified, or immunoprecipitated HDAC3, and from native, recombinant, or genetically modified HDAC3.

Components

The kit is sufficient for 100 assays in 96-well plates.

HDAC3 Assay Buffer (WM cap) Cat. No. EPI004A	25 mL
HDAC3 Substrate (Red cap) Cat. No. EPI004B	200 µL
HDAC3 Positive Control (Green cap) Cat. No. EPI004C	20 µL
AFC Standard (1 mM) (Yellow cap) Cat. No. EPI004D	100 µL
Developer (Orange cap) Cat. No. EPI004E	1 mL
Trichostatin A (HDAC3 Inhibitor) (Blue cap)Cat. No. EPI004F	200 µL

Reagents and Equipment Required

(but not provided)

96-well flat-bottom plate – It is recommended to use black sided, clear bottom plates for fluorescence assays.

Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. The HDAC assay buffer (EPI003A) can be stored at 4 °C. All other components should be stored at –20 °C, protected from light. All –20 °C reagents should be used within 2 months after thawing.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Read the entire protocol before performing the assay.

Procedure

All samples and standards should be run in duplicate.

Standard Curve Preparation

1. Prepare a 10 µM AFC (7-amino-4-trifluoromethyl coumarin) standard by mixing 10 µL of the 1 mM AFC Standard with 990 µL of dH_2O (1:100 dilution).

2. Add 0,1, 2, 4, 6, 8, 10 µL of 10 µM AFC into individual wells of a 96-well black sided, clear bottomed plate and adjust the volume to 100 µL/well with HDAC3 Assay Buffer to generate 0, 10, 20, 40, 60, 80, 100 pmole/well AFC standards.

Mix and read fluorometrically at $\lambda_{Ex}/\lambda_{Em}$ = 380/500 nm.

Sample Preparation

1. Prepare test samples in a black sided, clear bottomed 96-well plate in replicate, bringing each sample to 25 µL/well with HDAC3 Assay Buffer.
2. Add, to one of each sample replicates, 2 µL of Trichostatin A (HDAC3 Inhibitor) as background control. Make sure to include a positive set containing 2-5 µL HDAC3 adjusted to 25 µL volume with HDAC3 Assay Buffer, and one negative with 2-5 µL HDAC3 with 2 µL HDAC3 Inhibitor.
3. Mix well and incubate for 10 min at 37 °C.

Note: We suggest testing a range of sample aliquots to ensure your readings are within the standard curve linear range.

Substrate Preparation

For each test sample and background control, mix together:

HDAC3 Assay Buffer	23 µL
HDAC3 Substrate	2 µL

Add 25 µL substrate solution into each well **(Do Not add to Standard Curve wells).** Mix well.

Incubation

Incubate at 37 °C for 30 min

Developer

Once incubation is complete add 10 µL of developer and 40 µL of HDAC3 Assay Buffer into each well

(Do Not add to Standard Curve wells).

Mix well, incubate another 5 min at 37 °C to bring cleavage to a completion.

Measurement

Read $\lambda_{Ex}/\lambda_{Em}$ = 380/500 nm R_B and R_S for each background well and sample well, respectively.

Results

Calculations

Plot the AFC Standard Curve.
The RFU of fluorescence generated is $\Delta RFU = R_S - R_B$.
Apply the ΔRFU to the standard curve to get B pmole of AFC:

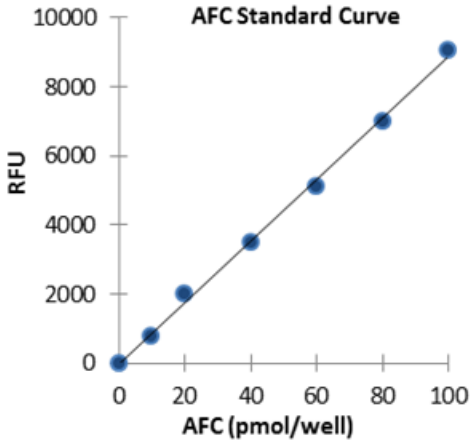
Activity = [B / (30 x V)] × Sample Dilution Factor = pmole/min/mL = µU/mL

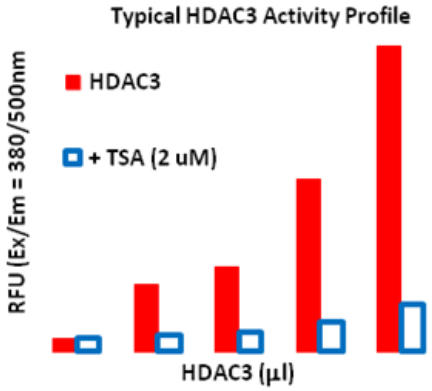
Where B is the AFC amount from the Standard Curve (in pmole).30 is the sample/substrate incubation time (in min).

V is the sample volume added into the reaction well (in mL).

Unit Definition

One unit is defined as the amount of HDAC3 able to generate 1.0 µmol of AFC per minute at 37 °C when incubated with the HDAC3 Substrate (R-H-K-K(Ac)-AFC).





Different amount of HDAC3 positive control were tested following kit protocol in the presence and absence of HDAC Inhibitor.

Troubleshooting Guide

Problems	Cause	Solution
Assay not working	Use of ice-cold assay buffer.	Assay buffer must be at room temperature.
	Omission of a step in the protocol.	Refer and follow the data sheet precisely.
	Plate read at incorrect wavelength.	Read Ex/Em = 380/500 nm
	Use of a different 96-well plate.	Fluorescence: Black plates (clear bottoms)
Samples with erratic readings	Use of an incompatible sample type.	Kit is suitable for nuclear extracts, purified, or immunoprecipitated HDAC3, and native, recombinant, or genetically modified HDAC3.
	Samples prepared in a different buffer.	Use the Assay Buffer provided in the kit.
	Samples used after multiple free-thaw cycles. HDAC Activity is lost after multiple freeze-thaw cycles.	Aliquot and freeze samples if needed for multiple assays. Avoid multiple freeze-thaw cycles.
	Presence of interfering substance in the sample.	Troubleshoot if needed.
	Use of old or inappropriately stored samples.	Use fresh samples or store at correct temperatures until use.
Lower/ Higher readings in Samples and Standards	Improperly thawed components.	Thaw all components completely and mix gently before use.
	Use of expired kit or improperly stored reagents.	Always check the expiration date and store the components appropriately.
	Allowing the reagents to sit for extended times on ice.	Always thaw and prepare fresh reaction mix before use.
	Incorrect incubation times or temperatures.	Refer to Steps 4 and 5.
	Incorrect volumes used.	Use calibrated pipettes and aliquot correctly.
Readings do not follow a linear pattern for Standard curve	Use of partially thawed components.	Thaw and resuspend all components before preparing the reaction mix.
	Pipetting errors in the standard.	Avoid pipetting small volumes.
	Pipetting errors in the reaction mix.	Prepare a master reaction mix whenever possible.
	Air bubbles formed in well.	Pipette gently against the wall of the tubes.
	Standard stock is at an incorrect concentration.	Use recommended Always refer the dilutions in the data sheet.

Problems	Cause	Solution
	Calculation errors	Recheck calculations after referring the data sheet.
	Substituting reagents from older kits/lots.	Use fresh components from the same kit.
Unanticipated results	Measured at incorrect wavelength.	Read Ex/Em = 380/500 nm
	Use of incompatible sample type.	Kit is suitable for nuclear extracts, purified, or immunoprecipitated HDAC3, and native, recombinant, or genetically modified HDAC3.
	Sample readings above/below the linear range.	Concentrate/Dilute sample so as to be in the linear range.
	Samples contain interfering substances.	Troubleshoot if it interferes with the kit.

Note: The most probable list of causes is under each problem section. Causes/Solutions may overlap with other problems.

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