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Product Information

Histone Deacetylase 8 (HDAC8) Activity Assay Kit

Catalog Number **EPI006**
Storage Temperature –20 °C

TECHNICAL BULLETIN

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 the Histone Deacetylase 8 (HDAC8) Activity Assay Kit, HDAC8 present in a test sample will act with the supplied Developer to deacetylate and then cleave the HDAC8 Substrate (R-H-K(Ac)-K(Ac)-AFC). This activity will release the fluorescent group, AFC, which can be detected at (λ_{ex} = 380/λ_{em} = 500 nm). Trichostatin A, an HDAC inhibitor, is included in the kit to verify HDAC8 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 HDAC8, and from native, recombinant, or genetically modified HDAC8.

Components

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

HDAC8 Assay Buffer (WM cap) Catalog Number EPI006A	25 mL
HDAC8 Substrate (Red cap) Catalog Number EPI006B	200 µL
HDAC8 Positive Control (Green cap) Catalog Number EPI006C	20 µL
AFC Standard (1 mM) (Yellow cap) Catalog Number EPI006D	100 µL
Developer (Orange cap) Catalog Number EPI006E	1 mL
Trichostatin A (HDAC3 Inhibitor, 100 µM) (Blue cap) Catalog Number EPI006F	200 µL

Reagents and equipment required but not provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms 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 (EPI006A) can be stored at 2–8 °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. Upon thawing aliquot HDAC8 Positive Control (EPI006C) and HDAC8 Substrate (EPI006B) and store at –20 °C, use within 2 months.

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.

<u>Standard Curve Preparation</u>

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 water (100-fold dilution).
2. Add 0, 2, 4, 6, 8, 10 µL of 10 µM AFC into individual wells of a 96 well black sided, clear well plate and adjust the volume to 100 µL/well with HDAC8 Assay Buffer to generate 0, 20, 40, 60, 80, 100 pmole/well AFC Standard.
3. Mix and read fluorometrically at (λ_{ex} = 380/λ_{em} = 500 nm).

Sample Preparation:

1. Prepare test samples in this same black sided, clear well plate, in replicate, to contain up to 50 µL/well in HDAC8 Assay Buffer.
2. Add 2 µL of HDAC8 Assay Buffer to one sample replicate and 2 µL of Trichostatin A (Inhibitor) to another sample replicate as the sample background control.
3. Use a positive control containing 1–2 µL of HDAC8, adjusting the volume to 50 µL with HDAC8 Assay Buffer.
4. Mix well and incubate for 10 minutes at 37 °C.

Notes: It is suggested to test a range of sample aliquots to ensure the readings are within the standard curve linear range.

DTT does not interfere with the assay.

Substrate Preparation

For each test sample and background control, prepare Substrate Solution (38 µL per well):

HDAC8 Assay Buffer	36 µL
HDAC8 Substrate	2 µL

Add 38 µL of Substrate Solution into each well (**Do Not** add to Standard Curve wells). Mix well.

Incubation

Incubate at 37 °C for 60 minutes.

Developer

Once incubation is complete add 10 µL of Developer into each well (**Do Not** add to Standard Curve wells).

Mix well and incubate another 5 minutes at 37 °C to bring cleavage to a completion.

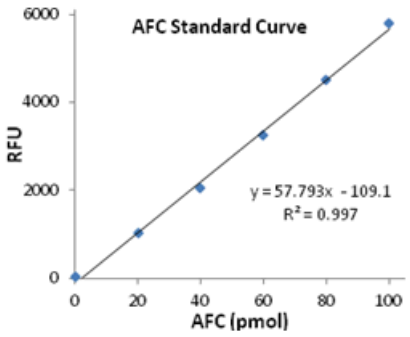
Measurement

Read (λ_{ex} = 380/λ_{em} = 500 nm) for each well, R_B (background) and R_S (sample).

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 pmole of AFC (B in the following equation):

Activity = [B/(60 × V)] × Sample Dilution Factor

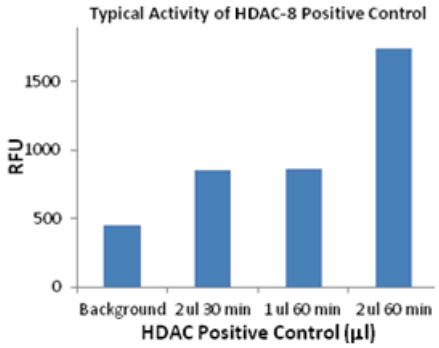
where:
B = the AFC amount from the Standard Curve (in pmole).

60 = the sample/substrate incubation time (in minutes).

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

HDAC8 Activity may be expressed as:
pmole/min/mL = µU/mL

Unit definition: One unit is defined as the amount of HDAC8 able to generate 1.0 µmole of AFC per minute at 37 °C when incubated with the HDAC8 Substrate (R-H-K(Ac)-K(Ac)-AFC).



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 bulletin precisely
	Plate read at incorrect wavelength	Read Ex/Em = 380/500 nm
	Use of a different 96-well plate	Fluorescence: black sided, clear well plate
Samples with erratic readings	Use of an incompatible sample type	Suitable for nuclear extracts, purified, or immunoprecipitated HDAC8, and native, recombinant, or genetically modified HDAC8.
	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 & 5 to verify correct incubation times and temperatures
	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	Always refer to the dilutions in the bulletin
	Calculation errors	Recheck calculations after referring to Step 7
	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
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Use of incompatible sample type	Suitable for nuclear extracts, purified, or immunoprecipitated HDAC8, and native, recombinant, or genetically modified HDAC8.
	Sample readings above/below the linear range	Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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