

Product Information

Histone Deacetylase 3 (HDAC3) Inhibitor Screening Kit

Catalog Number **EPI005**

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, HDACs play a crucial role in mammalian development and disease. HDACs are involved in important biological activities, such as cell differentiation, proliferation, apoptosis, and senescence.

Of the several classes of HDACs, this kit assays a member of the zinc-independent, NADH requiring class, HDAC3. With Sigma's HDAC3 Inhibitor Screening Kit, HDAC3 Enzyme acts with the supplied Developer to deacetylate and then cleave the HDAC3 Substrate [Arg-His-Lys-Lys(Ac)-AFC]. This activity releases the quenched fluorescent group, AFC, which can be detected at $\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 380/500\text{ nm}$. In the presence of a HDAC3 inhibitor, AFC is not released and its fluorescence remains quenched. The kit provides a rapid, simple, sensitive, and reliable test, suitable for either individual tests or high throughput screening of HDAC3 inhibitors. Trichostatin A (TSA) is included as a control inhibitor to compare with the efficacy of test inhibitors.

Components

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

HDAC3 Assay Buffer (WM cap) Catalog Number EPI005A	25 mL
HDAC3 Substrate (Red cap) Catalog Number EPI005B	200 μL
HDAC3 Enzyme (Green cap) Catalog Number EPI005C	200 μL
Developer (Orange cap) Catalog Number EPI005D	1 mL
Trichostatin A (HDAC3 Inhibitor) (Blue cap) Catalog Number EPI005E	50 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 Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store HDAC-3 enzyme (EPI005C) at -80°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. Allow Assay Buffer to warm to room temperature before use. Read the entire protocol before performing the assay.

Procedure

All samples and standards should be run in duplicate.

1. Enzyme solution

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 μL HDAC-3 solution.

48 μL HDAC3 Assay Buffer
2 μL HDAC-3 Enzyme

2. Sample Preparation: for all compounds to screen, inhibitor control and blank control

- Dissolve candidate compounds into a proper solvent, (specific for each inhibitor). Then make 4x serial dilutions in HDAC3 Assay Buffer for a final 75 μL volume.

- b. To make Trichostatin A Inhibitor Control, dilute 1:9 in HDAC3 Assay Buffer.
- c. Using a black sided, clear bottomed plate, add 25 μL of either diluted test inhibitors (test), diluted Trichostatin A Inhibitor (inhibition control) or HDAC3 Assay Buffer (no inhibitor control) with the above 50 μL HDAC3 enzyme mix. Mix well, and incubate for 10 minutes at 37 $^{\circ}\text{C}$.
- d. Blank well: Add 75 μL of HDAC3 Assay Buffer into a blank well as background.

3. Substrate Preparation

For each well, prepare a total 25 μL Substrate Solution

HDAC3 Assay Buffer	23 μL
HDAC3 Substrate	2 μL

Mix, initiate reaction with 25 μL of this substrate into each of the above sample wells from Step 2.

4. Incubation

Mix, incubate 60 min at 37 $^{\circ}\text{C}$.

5. Developer

Once incubation is complete add 10 μL of developer into each well including control

Mix well, incubate for 5 min at 37 $^{\circ}\text{C}$.

6. Measurement

Read $\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 380/500$ nm for relative fluorescent units (RFU) of background wells (R_{BG}), No Inhibitor control wells (R_{NI}), and test or inhibitor controls (R_{T}), respectively.

Results

7. Calculations

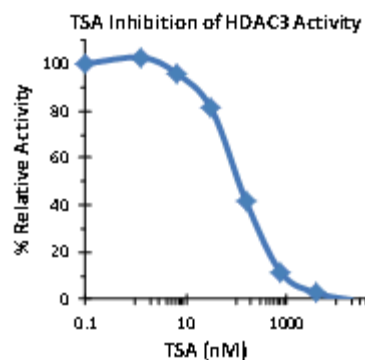
The percent inhibition is calculated taking the difference (ΔRFU) between the inhibited fluorescence ($R_{\text{T}} - R_{\text{BG}}$) and the maximum fluorescence ($R_{\text{NI}} - R_{\text{BG}}$) normalized to this maximum fluorescence

The equations are given below

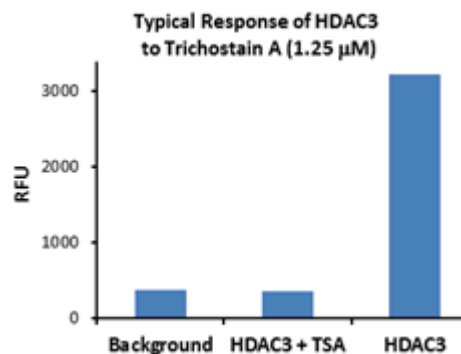
$$\text{Maximum Fluorescence} = \Delta\text{RFU EC} = (R_{\text{NI}} - R_{\text{BG}})$$

$$\text{Inhibited Fluorescence} = \Delta\text{RFU S} = (R_{\text{T}} - R_{\text{BG}})$$

$$\% \text{ inhibition} = \frac{[(\Delta\text{RFU EC} - \Delta\text{RFU S}) / \Delta\text{RFU EC}] \times 100\%}{}$$



Typical inhibition of HDAC3 activity with defined amounts of TSA



Inhibition of a known 1 unit of HDAC3 with TSA

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: Use a black sided, clear bottomed plate
	Samples prepared in a different buffer	Use the assay buffer provided in the kit
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	See 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
	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
	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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