HDAC3 Activity Fluorometric Assay Kit

(Catalog # BN00607; 100 assays; Store kit at -80°C)

I. Introduction:

Histone deacetylases (HDACs) represent a large family of enzymes identified as key regulators of nucleosomal histone acetylation, a major event that controls eukaryotic gene transcription and are classified into three groups. HDACs are transcriptional repressors with crucial roles in mammalian development. They are believed to be involved in important biological activities, such as cell differentiation, proliferation, apoptosis, and senescence. In Assay Genie's HDAC3 activity Kit, HDAC3 and Developer will deacetylate and cleave the substrate [R-H-K-K(Ac)-AFC] to release the AFC molecule, which can be detected fluorometrically (Ex/Em = 380/500 nm). The kit provides a rapid, simple, sensitive, and reliable test, which is also suitable for high throughput measurement of HDAC3 activity in purified, immunoprecipitated and recombinant or genetically modified HDAC-3 samples.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
HDAC3 Assay Buffer	25 ml	WM	BN00607-1
HDAC3 Substrate	200 µl	Red	BN00607-2
HDAC3 Positive Control	20 µl	Green	BN00607-3
AFC Standard (1 mM)	100 µl	Yellow	BN00607-4
Developer	1 ml	Orange	BN00607-5
Trichostatin A	200 µl	Blue	BN00607-6

III. Storage and Handling:

Remove the vial of HDAC3 and store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

HDAC3 Positive Control: Aliquot and store at -80°C. Use within 2 months

HDAC3 Substrate: Upon thawing aliquot and store at -20°C. Use within 2 months.

Developer and Trichostatin A: Store at -20°C; use within 2 months after first thaw.

HDAC3 Assay Buffer: Store at 4°C

IV. HDAC3 Activity Assay Protocol:

1. **Standard Curve Preparation:** 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. Add 0,1, 2, 4, 6, 8, 10 µl 10 µM AFC into individual wells in a 96-well plate and adjust the volume to 100 µl/well with HDAC3 Assay Buffer to generate 0, 10, 20, 40, 60, 80, 100 pmol/well of AFC Standard. Read fluorometrically at Em/Ex = 380/500 nm after mixing.
2. **Sample Preparations:** Prepare test samples as replicates of up to 25 µl/well with HDAC3 Assay Buffer in a 96-well plate. To one sample replicate and 2 µl HDAC3 Inhibitor (Trichostatin A) as background control. Use 2-5 µl HDAC3 as a positive control and adjust volume to 25 µl with HDAC3 Assay Buffer. Mix well and incubate for 10 min at 37°C. We suggest testing several doses of your sample to ensure the readings are within the standard curve linear range.
3. **Substrate Preparation:** For each test sample and background control:

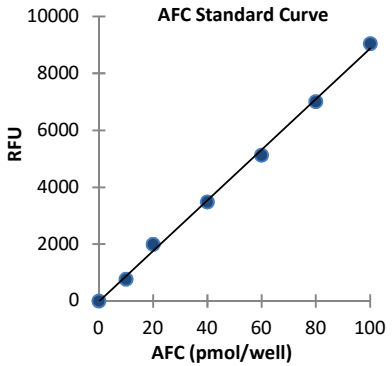
   23 µl HDAC3 Assay Buffer
   2 µl HDAC3 Substrate

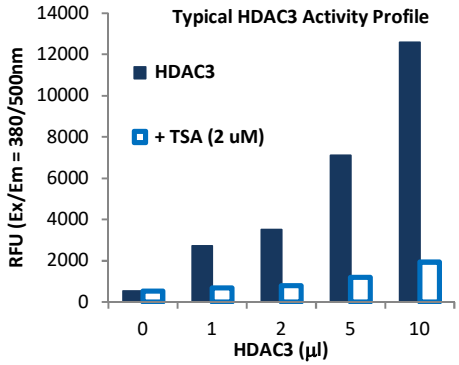
   Add 25 µl substrate solution into each well **(Do Not add to Standard Curve wells).** Mix well. Incubate at 37°C for 30 min.
5. **Developer**: Add 10 µl of developer and 40 µl HDAC3 Assay Buffer into each well **(Do Not add to Standard Curve wells)**. Mix well, incubate another 5 min at 37°C.
6. **Measurement:** Read Ex/Em = 380/500 nm R_B and R_S for each background well and sample well, respectively.
7. **Calculation:** Plot the AFC Standard Curve. The RFU of fluorescence generated is Δ RFU = R_S – R_B. Apply the Δ RFU to the standard curve to get B pmol of AFC:

   Activity = $\frac{B}{(30)\times V}$ **× Sample Dilution Factor = pmol/min/ml = U/ml**

   Where: B is the AFC amount from the Standard Curve (in pmol).
   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 pmol of AFC per minute at 37°C when incubated with the HDAC3 Substrate (R-H-K-K(Ac)-AFC).





FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL 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	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• 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 expiry 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 datasheet & 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 the dilutions in the data sheet
	• 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	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• 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.		