

RayBio® HDAC-3 Activity Assay Kit

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RayBio® HDAC-3 Activity Assay
Kit Protocol

(Cat#: 68AT-HDAC3-S100)



RayBiotech, Inc.

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TABLE OF CONTENTS

I. Introduction.....	1
II. Reagents.....	2
III. Storage and Stability.....	2
IV. Calpain Assay Procedure	2
V. General Troubleshooting Guide.....	4

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 RayBiotech'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. REAGENTS

Components	100 Assays	Cap Code	Part Number
HDAC3 Assay Buffer	25 ml	WM	Item A
HDAC3 Substrate	200 μ l	Red	Item B
HDAC3 Positive Control	20 μ l	Green	Item C
AFC Standard (1mM)	100 μ l	Yellow	Item D
Developer	1 ml	Orange	Item E
Trichostatin A	200 μ l	Blue	Item F

III. STORAGE AND STABILITY

- 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 ASSAY PROCEDURE

- 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 dH₂O. 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 $E_m/E_x = 380/500$ nm after mixing.
- 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

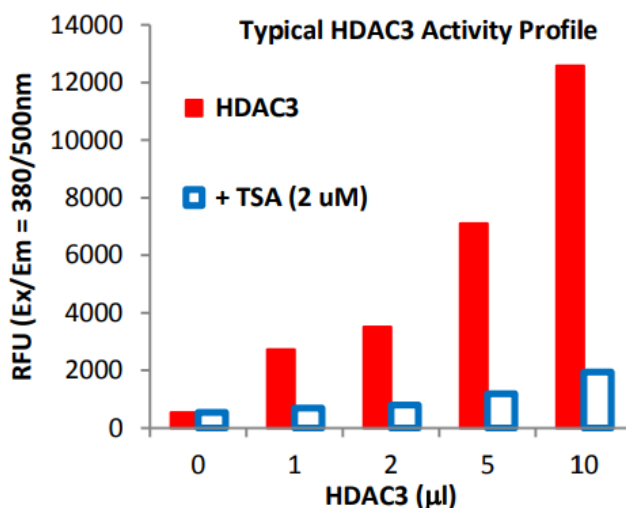
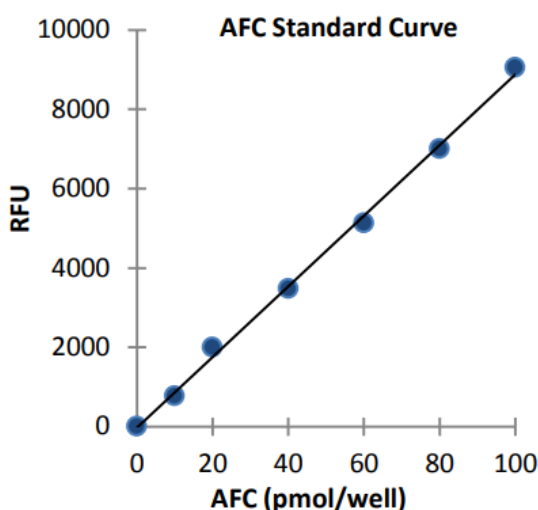
incubate for 10 min at 37°C. We suggest testing several doses of your sample to ensure the readings are within the standard curve linear range.

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.
4. **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.
5. **Measurement:** Read Ex/Em = 380/500 nm RB and RS for each background well and sample well, respectively.
6. **Calculation:** Plot the AFC Standard Curve. The RFU of fluorescence generated is $\Delta \text{RFU} = R_S - R_B$. Apply the ΔRFU to the standard curve to get B pmol of AFC.

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

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 37C when incubated with the HDAC3 Substrate (R-H-K-K(Ac)-AFC).



V. GENERAL TROUBLESHOOTING GUIDE

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/tissue samples were not completely homogenized • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions. • Use Dounce homogenizer (increase the number of strokes); observe the lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the substrate • Pipetting errors in the reaction mix • Air bubbles formed in well • Substrate stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
<p>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		

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