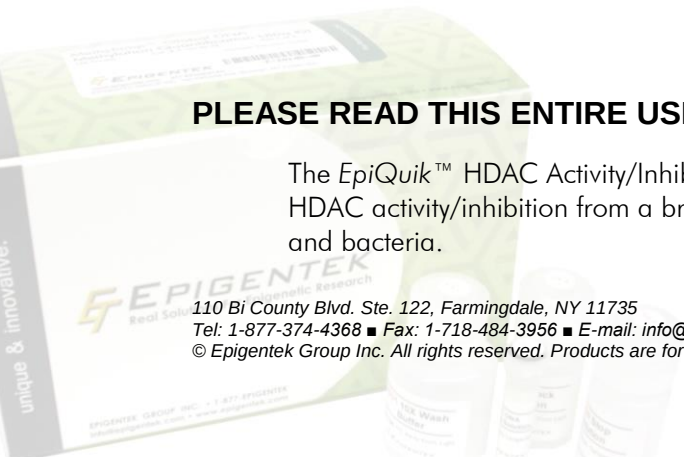
EpiQuik™ HDAC Activity/Inhibition Assay Kit (Fluorometric)

Base Catalog # P-4001



PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ HDAC Activity/Inhibition Assay Kit (Fluorometric) is very suitable for measuring HDAC activity/inhibition from a broad range of species, including mammalian cells/tissues, plants, and bacteria.

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KIT CONTENTS

Components	48 assays P-4001-48	96 assays P-4001-96
H1 (10X Wash Buffer)	14 ml	28 ml
H2 (HDAC Assay Buffer)	1.5 ml	3 ml
H3 (Biotinylated HDAC Substrate)*	50 µl	100 µl
H4 (HDAC Inhibitor, 0.5 mM)*	50 µl	100 µl
H5 (HDAC Assay Standard, 20 µg/ml)*	25 µl	50 µl
H6 (Capture Antibody, 1000 µg/ml)*	5 µl	10 µl
H7 (Detection Antibody, 200 µg/ml)*	10 µl	20 µl
Fluoro-Developer	3 ml	6 ml
8-Well Assay Strips (with Frame)	6	12
User Guide	1	1

** For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.*

SHIPPING & STORAGE

The kit is shipped in two parts: one part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store H3, H4, H5, and H7 at –20°C away from light; (2) Store H1, H6, Fluoro-Developer, and 8-Well Assay Strips at 4°C away from light; (3) Store H2 at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

Note: Check if wash buffer, H1, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Orbital shaker
- ☐ Pipettes and pipette tips
- ☐ Reagent reservoir
- ☐ Microplate reader
- ☐ 1.5 ml microcentrifuge tubes

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GENERAL PRODUCT INFORMATION

Usage Limitation: The *EpiQuik*™ HDAC Activity/Inhibition Assay Kit (Fluorometric) is for research use only and is not intended for diagnostic or therapeutic application.
Quality Control: Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design.

Intellectual Property: *EpiQuik*™ is a trademark of Epigentek, Inc.

A BRIEF OVERVIEW

Histone deacetylases (HDACs) play a critical role in transcriptional repression of the gene expression in eukaryotic cells through catalyzing the hydrolytic removal of acetyl groups from histone lysine residues. HDACs are tightly involved in cell cycle regulation, cell proliferation, and in development of human cancer. HDAC inhibition displays significant effects on apoptosis, cell cycle arrest, and differentiation in cancer cells. HDAC inhibitors are currently being developed as potential anticancer agents. There are several methods used for measuring HDAC activity/inhibition. However, most of these methods available so far are time consuming, laborious, produce radioactive waste, or cannot measure precise HDAC activity and inhibitory effects of inhibitors.

The *EpiQuik*™ HDAC Activity/Inhibition Assay Kit (Fluorometric) uses a proprietary and unique procedure to measure HDAC activity/inhibition with the following features:

- Fast procedure, which can be finished within 3 hours.
- Innovative fluorometric assay without the use of radioactivity, extraction, or chromatography.
- Direct measurement of HDAC activity and inhibition without the use of lysyl endopeptidase, thereby avoiding the false inhibitory effect on HDACs, and allowing more accurate measurement.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ HDAC Activity/Inhibition Assay Kit (Fluorometric) is designed for measuring total HDAC activity/inhibition. In an assay with this kit, the unique acetylated histone substrate is stably captured on the strip wells. Active HDACs bind to and deacetylate histone substrate. The remaining un-deacetylated substrate can be recognized with a high affinity acetylated histone antibody. The ratio or amount of the un-deacetylated histone, which is inversely proportional to HDAC enzyme activity, can then be fluorometrically quantified.

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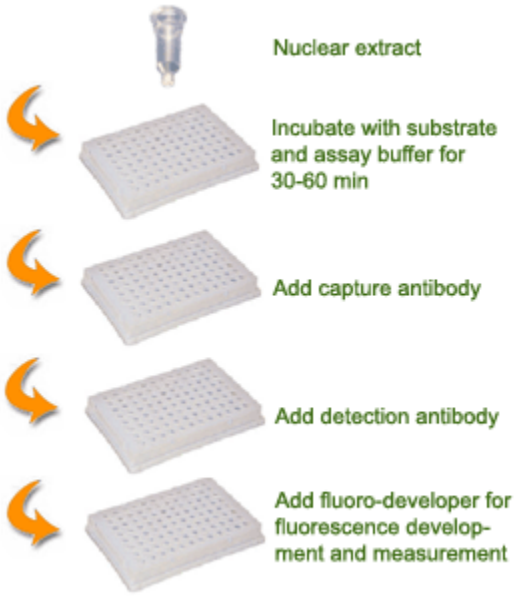


Schematic Procedure for Using the *EpiQuik*™ HDAC Activity/Inhibition Assay Kit (Fluorometric)

PROTOCOL

1. Prepare nuclear extracts by using you own successful method. For your convenience and the best results, Epigentek offers a nuclear extraction kit (Cat. No. OP-0002-1) optimized for use in the *EpiQuik*™ series. Nuclear extracts can be used immediately or stored at –80°C for future use.

2. Determine the number of strip wells required. Leave these strips in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Dilute **H1** with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (ex: 1 ml of **H1** + 9 ml of distilled water).

3. Dilute **H3** at a 1:50 ratio with **diluted H1**, and add 50 µl of the **diluted H3** into each sample well and each control well (control well is **H3** only). Do not add diluted **H3** to the wells for the blank and standard curve. For preparation of the standard curve, add 50 µl of **diluted H1** into the wells (without **H3** added), followed by adding 1 µl of **H5** at different amounts (0.1 – 10 ng). Cover the wells with Parafilm M and incubate at room temperature for 30-45 minutes.

4. Aspirate and wash each well with 150 µl of **diluted H1** two times.

5. Add 28 µl of **H2**, and 2 µl of nuclear extracts (4-20 µg) or HDAC enzymes to the strip wells, except the wells for the control, blank, and standard curve. For the control and standard curve, instead of nuclear extracts, add 2 µl of **H2**. For HDAC inhibition, add 2 µl of different amounts of **H4** or tested inhibitors, and reduce **H2** volume to 26 µl. For the blank, add 30 µl of **H2** into the wells. Mix, cover the strip wells, and incubate at 37°C for 45-60 minutes.

6. Aspirate and wash each well with 150 µl of **diluted H1** three times.

7. Dilute **H6** (at a 1:1000 ratio) to 1 μg/ml with **diluted H1**. Add 50 μl of the **diluted H6** to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).

8. Aspirate and wash each well with 150 μl of **diluted H1** four times.

9. Dilute **H7** (at a 1:1000 ratio) to 0.2 μg/ml with **diluted H1**. Add 50 μl of the **diluted H7** to each strip well and incubate at room temperature for 25-30 minutes.

10. Aspirate and wash each well with 150 μl of **diluted H1** five to six times.

11. Add 50 μl of the **Fluoro-Developer** into the wells and incubate at room temperature for 1-5 minutes away from light. The color in the standard wells containing the higher concentrations may turn slightly pink during this period. Measure and read fluorescence on a fluorescence microplate reader at $530_{EX}/590_{EM}$ nm.

    Note: *If the strip well frame does not fit the fluorescence reader, transfer the solution to a standard 96-well microplate and read fluorescence at $530_{EX}/590_{EM}$ nm.*

12. Calculate HDAC activity or inhibition. For simple calculation:

$$\text{HDAC activity (RFU/h/ug)} = \frac{[\text{RFU (control – blank)} - \text{RFU (sample – blank)}]}{\text{Reaction time (h) x protein amount added}}$$

$$\text{Inhibition \%} = \left(1 - \frac{[\text{RFU (control – blank)} - \text{RFU (inhibitor sample – blank)}]}{[\text{RFU (control – blank)} - \text{RFU (no inhibitor sample – blank)}]}\right) \times 100\%$$

For an accurate calculation, plot RFU value versus amount of **H5** and determine the slope as delta RFU/ng.

Calculate HDAC activity using the following formula:

$$\text{Activity (ng/h/ug)} = \frac{[\text{RFU (control-blank)} - \text{RFU (sample – blank)}]}{\text{slope x h x protein amount added}}$$

TROUBLESHOOTING

No Signal for the Sample

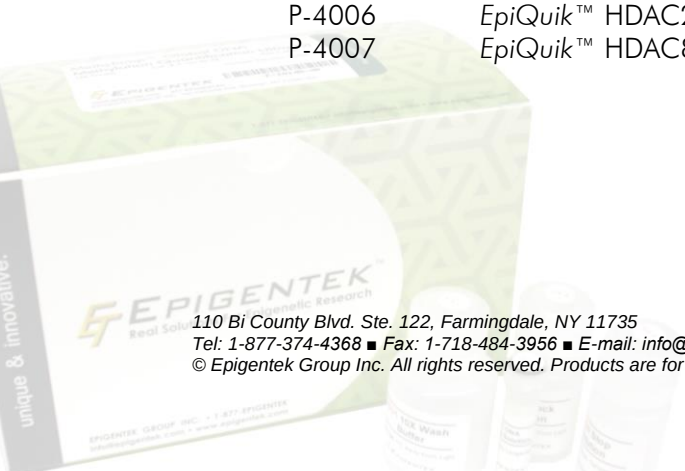
The protein sample is not properly extracted.	Ensure the protein extraction protocol is suitable for nuclear protein extraction.
The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of protein.
The sample is not prepared from fresh cells or tissues.	The nuclear extracts from frozen cells or tissues significantly lose enzyme activity. A fresh sample should be used.
Nuclear extracts are incorrectly stored.	Ensure the nuclear extracts are stored at –80°C.
Reagents are added incorrectly.	Check if reagents are added in order and if any steps of the procedure may have been omitted by mistake.
Incubation time and temperature are incorrect.	Ensure the incubation time and temperature described in the protocol are followed correctly.
Absence of HDAC activity in the sample due to treatment.	N/A.

High Background Present for the Blank

The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Overdevelopment.	Decrease development time in step 11.

RELATED PRODUCTS

P-4002	*EpiQuik*™ HDAC Activity/Inhibition Assay Kit (Colorimetric)
P-4005	*EpiQuik*™ HDAC1 Assay Kit
P-4006	*EpiQuik*™ HDAC2 Assay Kit
P-4007	*EpiQuik*™ HDAC8 Assay Kit



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