

# EpiQuik™ HDAC1 Assay Kit (Colorimetric)

Base Catalog # P-4005



## PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The EpiQuik™ HDAC1 Assay Kit (Colorimetric) is very suitable for measuring HDAC1 levels from various fresh tissues and cultured mammalian cells.

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## KIT CONTENTS

Contents	48 assays P-4005-48	96 assays P-4005-96
HA1 (10X Wash Buffer)	11 ml	22 ml
HA2 (HDAC Assay Buffer)	1 ml	2 ml
HA3 (Blocking Buffer)	10 ml	20 ml
HA4 (Capture Antibody, 200 µg/ml)*	13 µl	26 µl
HA5 (Detection Antibody, 200 µg/ml)*	10 µl	20 µl
HA6 (Developing Solution)	6 ml	12 ml
HA7 (Stop Solution)	3 ml	6 ml
HDAC1 Control (100 ng/µl)*	16 µl	32 µl
8-Well Assay Strip (with Frame)	6	12
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\* 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 the **HDAC1 Control** and **HA5** at -20°C; (2) Store **HA1**, **HA3**, **HA4**, **HA6**, and **8-Well Assay Strips** at 4°C away from light; (3) Store **all other components** at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.

**Note:** Check if wash buffer, **HA1**, 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
- ☐ Microplate reader
- ☐ 1.5 ml microcentrifuge tubes

## GENERAL PRODUCT INFORMATION

**Usage Limitation:** The EpiQuik™ HDAC1 Assay Kit (Colorimetric) is for research use only and is not intended for diagnostic or therapeutic application.

**Quality Control:** Epigenetek 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 Group 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. Three distinct families of HDACs have been described comprising a group of at least 20 proteins in humans. HDAC1 is a class I histone deacetylase containing 482 amino acid residues. HDAC1 has been shown to interact directly with transcription factors and has been shown to deacetylate histone proteins H3 and H4.

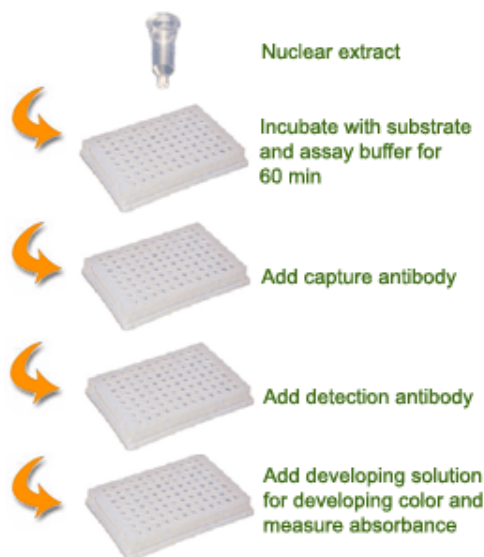
The major assay for measuring the expression or amount of HDAC1 protein currently is the Western blot. This method requires electrophoresis and transfer process, which makes the assay inconvenient, time consuming, and has low throughput. The *EpiQuik*™ HDAC1 Assay Kit (Colorimetric) addresses these problems by using a unique procedure to measure amount of HDAC1. The kit has following features:

- The fastest procedure, which can be finished within 3 hours.
- Innovative colorimetric assay to semi-quantitatively measure HDAC1 amount without the need of electrophoresis.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

## PRINCIPLE & PROCEDURE

The *EpiQuik*™ HDAC1 Assay Kit(Colorimetric) is designed for measuring total HDAC1 amount from tissues or cells. In an assay with this kit, the nuclear proteins containing HDAC1 are stably coated on the strip wells. The HDAC1 is recognized with a high-affinity specific antibody. The amount of HDAC1 can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.





Schematic Procedure for Using the *EpiQuik™* HDAC1 Assay Kit (Colorimetric).

## PROTOCOL

1. Prepare nuclear extracts by using your own successful method. For your convenience and best results, Epigentek offers a nuclear extraction kit (Cat. No. OP-0002-1) optimized for use in *EpiQuik™* series. Nuclear extracts can be used immediately or stored at  $-80^{\circ}\text{C}$  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  $4^{\circ}\text{C}$ ). Dilute **HA1** (10X Wash Buffer) with distilled water (pH 7.2-7.5) at a 1:10 ratio (ex: 1 ml of **HA1** + 9 ml distilled water) in order to create, **1X HA1**.
3. Adjust protein concentration to  $0.4\text{--}1\ \mu\text{g}/\mu\text{l}$  with **HA2** and add  $5\ \mu\text{l}$  ( $2\text{--}5\ \mu\text{g}$ ) of the protein solution into the central area of each well. Spread out the solution over the bottom of the strip well by pipetting the solution up and down several times. Incubate the strip wells at  $37^{\circ}\text{C}$  (with no humidity) for 60-90 minutes to evaporate the solution and completely dry the wells. For the blank, add  $5\ \mu\text{l}$  of **HA2** to the wells. For the positive control, dilute the **HDAC1 Control** to  $2\text{--}30\ \text{ng}/\mu\text{l}$  with **HA2** and add  $5\ \mu\text{l}$  ( $10\text{--}150\ \text{ng}$ ) of **diluted HDAC1 Control** solution to the wells.
4. Add  $150\ \mu\text{l}$  of **HA3** to the dried wells and incubate at  $37^{\circ}\text{C}$  for 30-45 minutes.
5. Aspirate and wash each well with  $150\ \mu\text{l}$  of **1X HA1** three times.
6. Dilute **HA4** (at a 1:200 ratio) to  $1\ \mu\text{g}/\text{ml}$  with **1X HA1**. Add  $50\ \mu\text{l}$  of **diluted HA4** to each assay strip well and 2 control strip wells. Incubate the samples at room temperature for 60 minutes on a orbital shaker (50-100 rpm).
7. Aspirate and wash each well with  $150\ \mu\text{l}$  of **1X HA1** four times.

8. Dilute the **HA5** (at 1:1000 ratio) to 0.2 µg/ml with **1X HA1**. Add 50 µl of **diluted HA5** to each strip well and incubate at room temperature for 30 minutes.
9. Aspirate and wash each well with 150 µl of **1X HA1** four times. Allow 3 minutes for the last wash.
10. Add 100 µl of **HA6** to each well and incubate at room temperature for 2-10 minutes away from light. Monitor the color development in the sample and standard wells (blue).
11. Add 50 µl of **HA7** to each well and read absorbance on microplate reader at 450 nm.
12. Calculate HDAC1 level:

$$\text{HDAC1 level (OD/ml)} = (\text{sample OD} - \text{blank OD}) \times \text{sample dilution}$$

For an accurate calculation, plot OD value versus amount of **HDAC1 Control** and determine the slope as delta OD/ng.

Calculate the amount of HDAC1 using the following formula:

$$\text{Amount (ng/mg protein)} = \frac{\text{OD (sample - blank)}}{\text{Slope X Protein amount (}\mu\text{g)}} \times 1000$$

*\*Nuclear extract added into sample wells at Step 3*

## TROUBLESHOOTING

### No Signal for Both the Positive Control and the Samples

Reagents are added incorrectly.

Check if reagents are added in the proper order and if any steps of the procedure may have been omitted by mistake.

The well is not completely dried.

Ensure the well is incubated without humidity and dried before adding **HA3** (Blocking Buffer).

The well is incorrectly washed before protein coating.

Ensure the well is not washed before adding positive control or protein extracts.

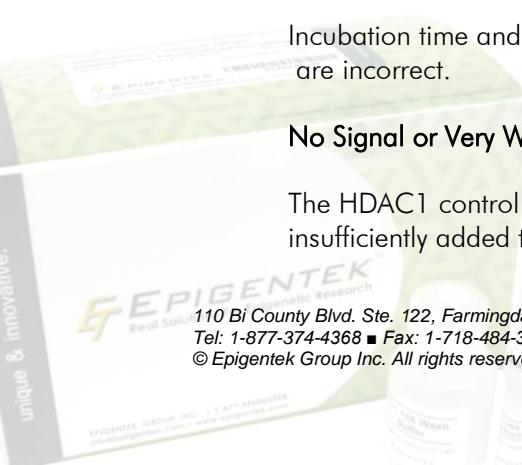
Incubation time and temperature are incorrect.

Ensure the incubation time and temperature described in the protocol are followed correctly.

### No Signal or Very Weak Signal For Only the Positive Control

The HDAC1 control protein is insufficiently added to the well.

Ensure a sufficient amount of control protein is added.



The positive control is degraded due to incorrect storage.

#### No Signal for Only the Sample

The protein amount is added into the well insufficiently.

Nuclear extracts are incorrectly stored.

#### High Background Present for the Blank

The well is not washed sufficiently.

Contaminated by the positive control.

Overdevelopment.

Follow the guidance in the protocol for storage of the positive control.

Ensure extract contains a sufficient amount of protein.

Ensure the nuclear extracts are stored at  $-80^{\circ}\text{C}$ .

Check if wash at each step is performed according to the protocol.

Ensure the well is not contaminated from adding the control protein or from using control protein contaminated tips.

Decrease development time in step 10.

## RELATED PRODUCTS

P-4002	<i>EpiQuik</i> <sup>™</sup> HDAC Activity/Inhibition Assay Kit (Colorimetric)
P-4006	<i>EpiQuik</i> <sup>™</sup> HDAC2 Assay Kit
P-4007	<i>EpiQuik</i> <sup>™</sup> HDAC8 Assay Kit

