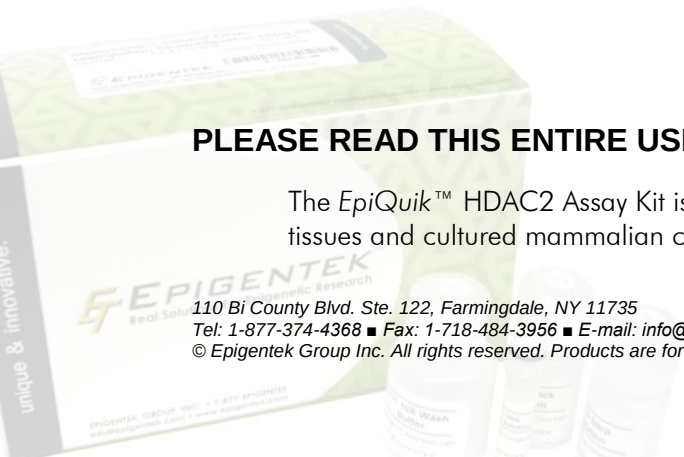
EpiQuik™ HDAC2 Assay Kit (Colorimetric)

Base Catalog # P-4006



PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ HDAC2 Assay Kit is very suitable for measuring HDAC2 levels from various fresh tissues and cultured mammalian cells.

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KIT CONTENTS

Contents	48 assays P-4006-48	96 assays P-4006-96
HB1 (10X Wash Buffer)	11 ml	22 ml
HB2 (HDAC Assay Buffer)	1 ml	2 ml
HB3 (Blocking Buffer)	10 ml	20 ml
HB4 (Capture Antibody, 200 µg/ml)*	13 µl	26 µl
HB5 (Detection Antibody, 200 µg/ml)*	10 µl	20 µl
HB6 (Developing Solution)	6 ml	12 ml
HB7 (Stop Solution)	3 ml	6 ml
HDAC2 Control (100 ng/µl)	16 µl	32 µl
8-Well Assay Strip (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: the first part at ambient room temperature, and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store HB5 and HDAC2 Control at –20°C; (2) Store HB1, HB3, HB4, HB6, 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, HB1, 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

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.

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Usage Limitation: The *EpiQuik*™ HDAC2 Assay Kits are for research use only and are not intended for diagnostic or therapeutic application.

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

A BRIEF OVERVIEW

Histone deacetylases (HDACs) play a critical role in transcriptional repression of 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 the 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. HDAC2 is a class I histone deacetylase containing 488 amino acid residues. HDAC2 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 HDAC2 protein currently is Western blot. This method requires electrophoresis and transfer process, which makes the assay inconvenient, time consuming, and has low throughput. The *EpiQuik*™ HDAC2 Assay Kit addresses these problems by using a unique procedure to measure the amount of HDAC2. The kit has the following features:

- The fastest procedure, which can be finished within 3 hours.
- Innovative colorimetric assay to semi-quantitatively measure HDAC2 amount without the need for electrophoresis.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ HDAC2 Assay Kit is designed for measuring total HDAC2 amount from tissues or cells. In an assay with this kit, the nuclear proteins containing HDAC2 are stably coated on the strip wells. The HDAC2 is recognized with a high-affinity specific antibody. The amount of HDAC2 can be quantified through an HRP conjugated secondary antibody color development system and is proportional to the intensity of the color development.

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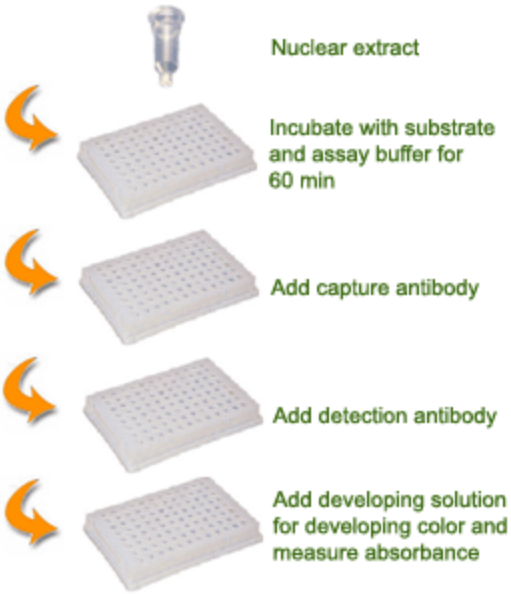

Schematic Procedure for Using the *EpiQuik*™ HDAC2 Assay Kit

PROTOCOL

1. Prepare nuclear extracts by using your 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 (the strip wells can be broken off). Leave these strip wells in the plate frame (remaining unused strips can be placed back in the bag. Seal the bag tightly and store at 4°C). Dilute **HB1** with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (ex: 1 ml of **HB1** + 9 ml of distilled water), in order to create **1X HB**.

3. Adjust protein concentration to 0.4-1 µg/µl with **HB2** and add 10 µl (4-10 µg) of the protein solution into the central area of each well. Spread the solution out over the bottom of the strip wells by pipetting the solution up and down several times. Incubate the strip wells at 37°C (without humidity) for 90 minutes to evaporate the solution and completely dry the wells. For the blank, add 5 µl of **HB2** to the wells. For the positive control, dilute **HDAC2 Control** to 1-20 ng/µl with **HB2** and add 10 µl (10-200 ng) of the **diluted HDAC2 Control** solution to the wells.

4. Add 150 µl of **HB3** to the dried wells and incubate at 37°C for 30-45 minutes.

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5. Aspirate and wash each well three times with 150 µl of **1X HB1** each time.

6. Dilute **HB4** (at a 1:200 ratio) to 1 µg/ml with **1X HB1**. Add 50 µl of **diluted HB4** to each well. Incubate the samples at room temperature for 60 minutes on a orbital shaker (50-100 rpm).

7. Aspirate and wash each well four times with 150 µl of **1X HB1** each time.

8. Dilute **HB5** (at a 1:1000 ratio) to 0.2 µg/ml with **1X HB1**. Add 50 µl of **diluted HB5** to each strip well and incubate at room temperature for 30 minutes.

9. Aspirate and wash each well four times with 150 µl of **1X HB1** each time. In the last wash, allow **1X HB1** to sit in the wells for 3 minutes before finally aspirating.

10. Add 100 µl of **HB6** 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 until it starts turning medium blue.

11. Add 50 µl of **HB7** to each well and read absorbance on microplate reader at 450 nm.

12. Calculate HDAC2 level:

$$\text{HDAC2 level (OD/ml)} = (\text{sample OD} - \text{blank OD}) \times \text{sample dilution}$$

For an accurate calculation, plot OD value versus amount of **HDAC2 control** and determine the slope as delta OD/ng.

Calculate the amount of HDAC2 using the following formula:

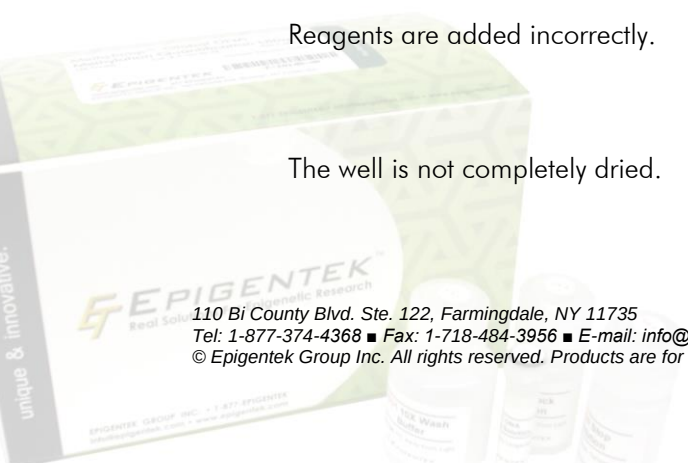
$$\text{Amount (ng/mg protein)} = \frac{\text{OD (sample} - \text{blank)}}{\text{Slope X Protein amount } (\mu g)^*} \times 1000$$

**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 in 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 **HB3** (Blocking Buffer).



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The well is incorrectly washed before protein coating.	Ensure the well is not washed before adding the 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 HDAC2 control protein is insufficiently added to the well.	Ensure sufficient amount of control protein is added.
The positive control is degraded due to incorrect storage.	Follow the guidance in the protocol for storage of the positive control.

No Signal for Only the Sample

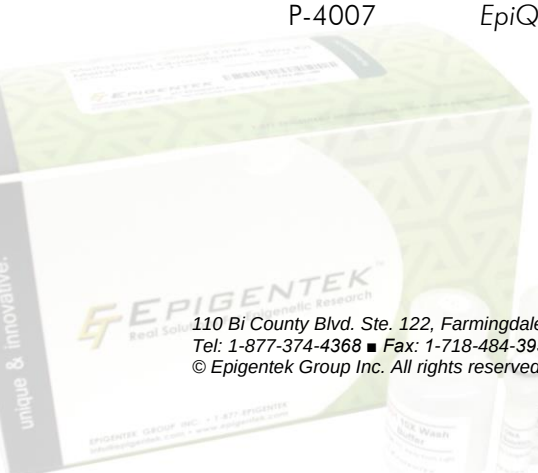
The protein amount is added into the well insufficiently.	Ensure the extract contains a sufficient amount of protein.
Nuclear extracts are incorrectly stored.	Ensure the nuclear extracts are stored at –80°C.

High Background Present for the Blank

The well is not washed enough.	Check if wash at each step is performed according to the protocol.
Contaminated by the positive control.	Ensure the well is not contaminated from add ing the control protein or from using control protein contaminated tips.
Overdevelopment.	Decrease development time in step 10.

RELATED PRODUCTS

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



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