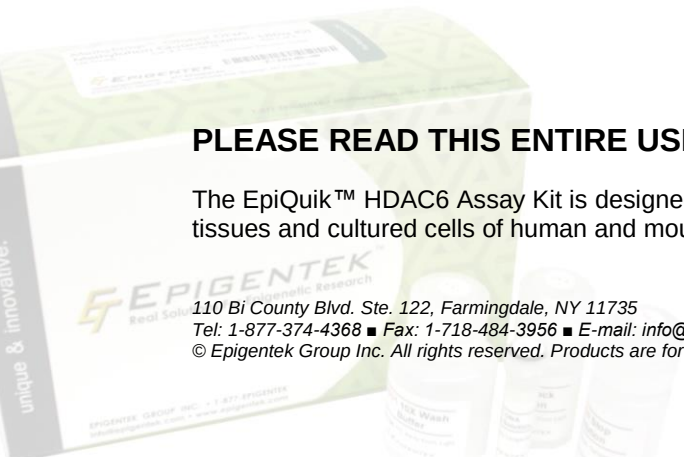
# EpiQuik™ HDAC6 Assay Kit (Colorimetric)

Base Catalog # P-4046



## PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The EpiQuik™ HDAC6 Assay Kit is designed for measuring HDAC6 protein amounts quantitatively from fresh tissues and cultured cells of human and mouse.

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## KIT CONTENTS

| Component | 48 Assays<br>Cat. #P-4046-48 | 96 Assays<br>Cat. #P-4046-96 | Storage<br>Upon Receipt |
|---|---|---|---|
| **WB** (10X Wash Buffer) | 12 ml | 25 ml | 4°C |
| **AB** (Assay Buffer) | 5 ml | 10 ml | 4°C |
| **BB** (Blocking Buffer) | 10 ml | 20 ml | 4°C |
| **CA** (Capture Antibody, 200 µg/ml)* | 13 µl | 26 µl | 4°C |
| **DA** (Detection Antibody, 200 µg/ml)* | 6 µl | 10 µl | –20°C |
| **ES** (Enhancer Solution)* | 6 µl | 10 µl | –20°C |
| **DS** (Developing Solution) | 6 ml | 12 ml | 4°C |
| **SS** (Stop Solution) | 6 ml | 11 ml | RT |
| **HDAC6 control (**200 µg/ml)* | 8 µl | 16 µl | –20°C |
| 8-Well Assay Strips (With Frame) | 6 | 12 | 4°C |
| User Guide | 1 | 1 | RT |

**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 **DA**, **ES**, and **HDAC6 Control** at –20°C away from light; (2) Store **WB**, **AB**, **BB**, **CA**, **DS**, and the **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: (1) Check if wash buffer, **WB**, contains salt precipitates before using. If so, warm (at room temperature or 37°C) and shake the buffer until the salts are re-dissolved; (2) check if a blue color is present in **DS** (Developing Solution), which would indicate contamination of the solution and should not be used. To avoid contamination, transfer the amount of **DS** required into a secondary container (tube or vial) before adding **DS** into the assay wells.*

## MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Adjustable pipette or multiple-channel pipette
- ☐ Multiple-channel pipette reservoirs
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Incubator for 37°C incubation

- ☐ Distilled water
- ☐ Nuclear extracts or purified HDAC6 enzyme
- ☐ Parafilm M or aluminum foil

## GENERAL PRODUCT INFORMATION

**Quality Control:** Each lot of the EpiQuik™ HDAC6 Assay Kit is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

**Product Warranty:** If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

**Safety:** Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

**Product Updates:** Epigentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

**Usage Limitation:** The EpiQuik™ HDAC6 Assay Kit is for research use only and is not intended for diagnostic or therapeutic applications.

**Intellectual Property:** The EpiQuik™ HDAC6 Assay Kit and methods of use contain proprietary technologies by Epigentek.

## A BRIEF OVERVIEW

Histone deacetylases (HDACs) play a critical role in transcriptional repression of gene expression in eukaryotic cells by 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 anti-cancer agents. Three distinct families of HDACs have been described, comprising a group of at least 20 proteins in humans. HDAC6 is a class II histone deacetylase containing 1215 amino acid residues. HDAC6 has two HDAC domains and deacetylates, histone and tubulin, suggesting it plays important roles in the nuclei and cytoplasm. HDAC6 regulates many biological processes including cell cycle progression.

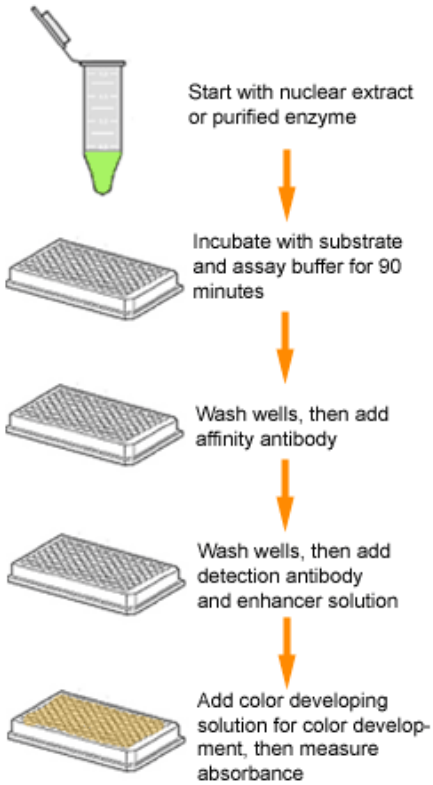
Western blot is currently the most prominent assay technique for measuring the expression or amount of HDAC6 protein. Yet this traditional method requires electrophoresis and transfer processes, which make the assay inconvenient, time consuming, and low throughput. The *EpiQuik*™ HDAC6 Assay Kit addresses these problems by using a unique procedure to measure the amount of HDAC6 proteins. The kit has the following features:

- Very fast procedure, which can be finished within 4 hours.
- Innovative colorimetric assay to quantitatively measure HDAC6 protein amount without the need for electrophoresis.

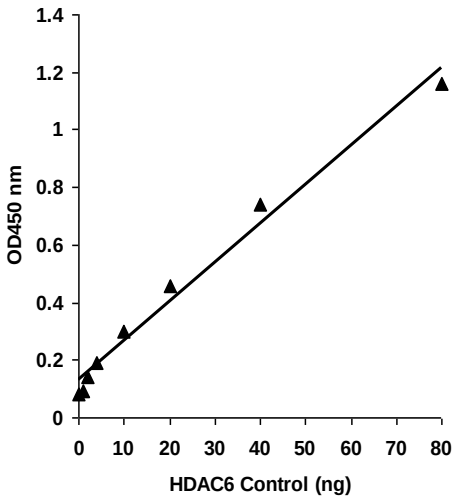
- High sensitivity and specificity – HDAC6-specific detection with detection limit as low as 1 ng of HDAC6 protein.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- HDAC6 control is included, which allows for the HDAC6 protein amount of the sample to be properly quantified.
- Simple, reliable, and consistent assay conditions.

## PRINCIPLE & PROCEDURE

The EpiQuik™ HDAC6 Assay Kit (Colorimetric) is designed for measuring total HDAC6 protein amount from tissues or cells. In an assay with this kit, the unique HDAC affinity substrate is stably coated on the strip well. The sample is added into the well and HDAC6 proteins contained in the sample bind to the substrate. The bound HDAC6 can be recognized with a HDAC6-specific antibody and colorimetrically quantified through an ELISA-like reaction. The amount of HDAC6 is proportional to the intensity of color development.




Schematic procedure of the EpiQuik™ HDAC6 Assay Kit (Colorimetric).




Illustrated standard curve generated with HDAC6 control

## ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

**Starting Materials**

*Input Amount:* The amount of nuclear extracts for each assay can be between 0.5 µg and 10 ug with an optimal range of 2 to 4 µg.

*Nuclear Extraction:* You can use your method of choice for preparing nuclear extracts from the treated and untreated samples. Epigentek also offers a nuclear extraction kit (Cat # OP-0002) optimized for use with this kit (see "Ordering Information").

Nuclear extracts should be stored at –80°C in aliquots until use.

**1. Working Buffer and Solution Preparation**

a. Prepare **Diluted WB** 1X Wash Buffer:

48-Assay Kit: Add 13 ml of **WB** 10X Wash Buffer to 117 ml of distilled water and adjust pH to 7.2-7.5.

96-Assay Kit: Add 26 ml of **WB** 10X Wash Buffer to 234 ml of distilled water and adjust pH to 7.2-7.5.

This **Diluted WB** 1X Wash Buffer can now be stored at 4°C for up to six months.

b. Prepare **Diluted CA** Capture Antibody Solution:

Dilute **CA** Capture Antibody with **Diluted WB** 1X Wash Buffer at a ratio of 1:200 (i.e., add 1 µl of **CA** to 200 µl of **Diluted WB**). 50 µl of **Diluted CA** will be required for each assay well.

c. Prepare **Diluted DA** Detection Antibody Solution:

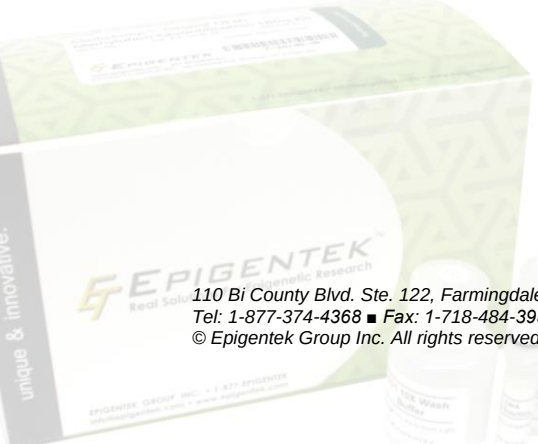
Dilute **DA** Detection Antibody with **Diluted WB** 1X Wash Buffer at a ratio of 1:2000 (i.e., add 1 µl of **DA** to 2000 µl of **Diluted WB**). 50 µl of **Diluted DA** will be required for each assay well.

d. Prepare **Diluted ES** Enhancer Solution:

Dilute **ES** Enhancer Solution with **Diluted WB** at a ratio of 1:5000 (i.e., add 1 µl of **ES** to 5000 µl of **Diluted WB**). About 50 µl of this **Diluted ES** will be required for each assay well.

e. Prepare **Diluted HDAC6 Control Standard**:

Suggested Standard Curve Preparation: First, dilute **HDAC6 control** with **AB** to a concentration of 40 ng/µl by adding 2 µl of **HDAC6 control** to 8 µl of **AB.** Then, further prepare concentration points of 1, 2, 4, 10, 20, and 40 ng/µl according to the following chart:



| Tube | HDAC6 (40 ng/µl) | AB | Resulting HDAC6 Concentration |
|---|---|---|---|
| 1 | 0.5 µl | 19.5 µl | 1 ng/µl |
| 2 | 0.5 µl | 9.5 µl | 2 ng/µl |
| 3 | 0.5 µl | 4.5 µl | 4 ng/µl |
| 4 | 1.5 µl | 4.5 µl | 10 ng/µl |
| 5 | 2.5 µl | 2.5 µl | 20 ng/µl |
| 6 | 5.0 µl | 0.0 µl | 40 ng/µl |

*Note: Keep each of the diluted solutions except **WB** 1X Wash Buffer on ice until use. Any remaining diluted solutions other than **Diluted WB** should be discarded if not used within the same day. The lower concentration point (ex: 0.5 ng/µl) can be also added if needed.*

**2. HDAC6 Binding**

a. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include blank and positive controls) to ensure that the signal generated is validated. Carefully remove un-needed strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C).

b. Blank Wells: Add 100 µl of **AB** to each blank well.

c. Standard Wells: Add 99 µl of **AB** and 2 µl of **Diluted HDAC6 control** to each standard well with a minimum of five wells, each at a different concentration between 1 and 20 ng/µl (based on the dilution chart in Step 1e; see Table 2 in the "Suggested Strip Well Setup" section as an example).

d. Sample Wells: Add 94 to 98 µl of **AB** and 2 to 6 µl of your nuclear extracts to each sample well. Total volume should be 100 µl per well.

*Note: (1) Follow the diagram in the "Suggested Strip Well Setup" section; (2) It is recommended to use 2 µg to 4 µg of nuclear extract per well.*

e. Cover strip-well microplate with Parafilm M or aluminum foil to avoid evaporation and incubate at 37°C for 90 to 120 min.

f. Remove the reaction solution from each well. Add 150 µl of **BB** Blocking Buffer to each well, then cover with Parafilm M or aluminum foil and incubate at 37°C for 30 min.

g. Remove the reaction solution from each well. Wash each well three times with 150 µl of the **Diluted WB** 1X Wash Buffer each time.

**3. Antibody Binding & Signal Enhancing**

a. Add 50 µl of the **Diluted CA** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 60 min.

b. Remove the **Diluted CA** solution from each well.

c. Wash each well three times with 150 µl of the **Diluted WB** each time.

d. Add 50 µl of the **Diluted DA** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

e. Remove the **Diluted DA** solution from each well.

f. Wash each well four times with 150 µl of the **Diluted WB** each time.

g. Add 50 µl of the **Diluted ES** to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 30 min.

h. Remove the **Diluted ES** solution from each well.

i. Wash each well five times with 150 µl of the **Diluted WB** each time.

*Note: Ensure any residual wash buffer in the wells is removed as much as possible at each wash step.*

## 4. Signal Detection

a. Add 100 µl of **DS** to each well and incubate at room temperature for 1 to 10 min away from light. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient HDAC6 protein.

b. Add 100 µl of **SS** to each well to stop enzyme reaction when color in the positive control wells turns medium blue. The color will change to yellow after adding **SS** and the absorbance should be read on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

*Note: (1) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs; (2) If the strip-well microplate frame does not fit in the microplate reader, transfer the solution to a standard 96-well microplate.*

## 5. HDAC6 Calculation

a. Calculate the average duplicate readings for the sample wells and blank wells.

b. Calculate % HDAC6 change using the following formula:

$$\textit{HDAC6 change \%} = \frac{\textit{Treated (Tested) Sample OD} - \textit{Blank OD}}{\textit{Untreated (Control) Sample OD} - \textit{Blank OD}} \times 100\%$$

Example calculation:

Average OD450 of treated sample is 0.5
Average OD450 of untreated control is 0.9
Average OD450 of blank is 0.1

$$\text{HDAC6 change \%} = \frac{(0.5 - 0.1)}{0.9 - 0.1} \times 100\% = 50\%$$

For Detailed Quantification :

1. Generate a standard curve and plot OD value versus amount of **HDAC6 control standard** at each concentration point.

2. Determine the slope as OD/ng (you can use *Microsoft Excel* statistical functions for slope calculation), then calculate the amount of HDAC6 using the following formula:

$$HDAC6\ (ng/mg\ protein) = \frac{(Sample\ OD - Blank\ OD)}{Slope \times Protein\ Amount\ (ug^*)} \times 1000$$

** Nuclear extract added into sample wells at Step 2d.*

## SUGGESTED BUFFER AND SOLUTION SETUP

**Table 1**. Approximate amount of required buffers and solutions for defined assay wells based on the protocol.

| Reagents | 1 well | 1 strip (8 wells) | 2 strips (16 wells) | 6 strips (48 wells) | 12 strips (96 wells) |
|---|---|---|---|---|---|
| **Diluted WB** | 2.5 ml | 20 ml | 40 ml | 120 ml | 240 ml |
| **AB** | 100 µl | 800 µl | 1600 µl | 4900 µl | 9600 µl |
| **BB** | 0.15 ml | 1.2 ml | 2.5 ml | 7.5 ml | 14.5 ml |
| **HDAC6 Control** | N/A | N/A | 2 µL (optional) | 4 µl | 4 µl |
| **Diluted CA** | 50 µl | 400 µl | 800 µl | 2400 µl | 4800 µl |
| **Diluted DA** | 50 µl | 400 µl | 800 µl | 2400 µl | 4800 µl |
| **Diluted ES** | 50 µl | 400 µl | 800 µl | 2400 µl | 4800 µl |
| **DS** | 0.1 ml | 0.8 ml | 1.6 ml | 4.8 ml | 9.6 ml |
| **SS** | 0.1 ml | 0.8 ml | 1.6 ml | 4.8 ml | 9.6 ml |

## SUGGESTED STRIP WELL SETUP

**Table 2**. The suggested strip-well plate setup for HDAC6 quantification in a 48-assay format (in a 96-assay format, Strips 7 to 12 can be configured as Sample). The controls and samples can be measured in duplicate.

| Well # | Strip 1 | Strip 2 | Strip 3 | Strip 4 | Strip 5 | Strip 6 |
|---|---|---|---|---|---|---|
| **A** | Blank | Blank | Sample | Sample | Sample | Sample |
| **B** | HDAC6 2 ng | HDAC6 2 ng | Sample | Sample | Sample | Sample |
| **C** | HDAC6 4 ng | HDAC6 4 ng | Sample | Sample | Sample | Sample |
| **D** | HDAC6 8 ng | HDAC6 8 ng | Sample | Sample | Sample | Sample |
| **E** | HDAC6 20 ng | HDAC6 20 ng | Sample | Sample | Sample | Sample |
| **F** | HDAC6 40 ng | HDAC6 40 ng | Sample | Sample | Sample | Sample |
| **G** | HDAC6 80 ng | HDAC6 80 ng | Sample | Sample | Sample | Sample |
| **H** | Sample | Sample | Sample | Sample | Sample | Sample |

## TROUBLESHOOTING

| Problem | Possible Cause | Suggestion |
|---|---|---|
| No signal or weak signal in both the positive control and sample wells | Reagents are added incorrectly. | Check if reagents are added in the proper order with the right amount, and if any steps in the protocol 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. |
| | Incorrect absorbance reading. | Check if appropriate absorbance wavelength (450 nm) is used. |
| | Kit was not stored or handled properly. | Ensure all components of the kit were stored at the appropriate temperature and the cap is tightly capped after each opening or use. |
| No signal or weak signal in only the standard curve wells | The standard amount is insufficiently added to the well in Step 2c. | Ensure a sufficient amount of standard is added. |
| | The standard is degraded due to improper storage conditions. | Follow the Shipping & Storage guidance in this User Guide for storage of **HDAC6 control.** |
| High background present in the blank wells | Insufficient washing of wells. | Check if washing recommendations at each step is performed according to the protocol. |
| | Contaminated by sample or standard. | Ensure the well is not contaminated from adding sample or standard accidentally or from using contaminated tips. |
| | Incubation time with **Diluted DA** is too long. | The incubation time at Step 3d should not exceed 90 min. |
| | Over-development of color. | Decrease the development time in Step 4a before adding **SS** Stop Solution in Step 4b. |
| No signal or weak signal only in sample wells | Protein sample is not properly extracted or purified. | Ensure your protocol is suitable for histone protein extraction. For the best results, it is advised to use Epigentek's Nuclear extraction Kit (Cat. No. OP-0002). |
| | Sample amount added into the wells is insufficient. | Ensure a sufficient amount of nuclear extracts is used as indicated in Step 2. The sample can be titrated to determine the optimal amount to use in the assay. |
| | Sample was not stored properly or has been stored for too long. | Ensure sample is stored in aliquots at –80°C, with no more than 6 months nuclear extracts. |

| | | |
|---|---|---|
| | Little or no HDAC6 in the sample. | This problem may be a result of many factors. If the affecting factors cannot be determined, use new or re-prepared nuclear extracts. |
| Uneven color development | Insufficient washing of the wells. | Ensure the wells are washed according to the guidance of washing and residue washing buffer is removed as much as possible. |
| | Delayed color development or delayed stopping of color development in the wells. | Ensure color development solution or stop solution is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well G or from well 1 to well 12). |

## RELATED PRODUCTS

**Nuclear Extract Preparation**

OP-0002 EpiQuik™ Nuclear Extraction Kit

**HDAC Activity/Inhibition Assay**

P-4034 Epigenase™ HDAC Activity/Inhibition Direct Assay Kit
P-4035 Epigenase™ HDAC Activity/Inhibition Direct Assay Kit (Fluorometric)
P-4036 Epigenase™ Universal SIRTActivity/Inhibition Assay Kit
P-4037 Epigenase™ Universal SIRT Activity/Inhibition Assay Kit (Fluorometric)

**HDAC Level Quantification Kit**

P-4005 EpiQuik™ HDAC1 Assay Kit
P-4006 EpiQuik™ HDAC2 Assay Kit
P-4040 EpiQuik™ HDAC3 Assay Kit
P-4042 EpiQuik™ HDAC4 Assay Kit
P-4046 EpiQuik™ HDAC6 Assay Kit
P-4048 EpiQuik™ HDAC7 Assay Kit
P-4007 EpiQuik™ HDAC8 Assay Kit
P-4050 EpiQuik™ HDAC9 Assay Kit
P-4052 EpiQuik™ HDAC10 Assay Kit
P-4054 EpiQuik™ HDAC11 Assay Kit

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