DATA SHEET

EpiQuik™ HDAC2 Assay Kit
Catalog No. P-4006

INTRODUCTION

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 AND 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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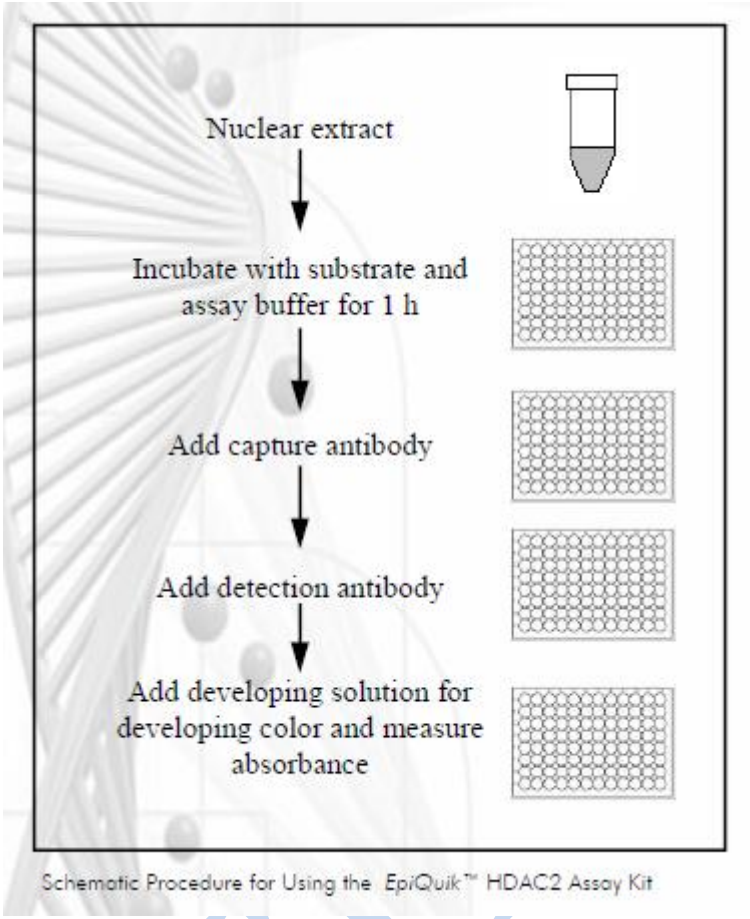

Schematic Procedure for Using the *EpiQuik™* HDAC2 Assay Kit

PRODUCT USE INFORMATION

The EpiQuik™ HDAC2 Assay Kit is very suitable for measuring HDAC2 levels from various fresh tissues and cultured mammalian cells.
The EpiQuik™ HDAC2 Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.
Epigentek guarantees the performance of all products in the manner described in our product instructions.
Epigentek reserves the right to change or modify any product to enhance its performance and design.
The EpiQuik™ HDAC2 Assay Kits are for research use only and are not intended for diagnostic or therapeutic application.
EpiQuik™ is a trademark of Epigentek Group Inc.

KIT CONTENTS

Contents	24 assays P-4006-24	48 assays P-4006-48	96 assays P-4006-96
HB1 (10X Wash Buffer)	6 ml	11 ml	22 ml
HB2 (HDAC Assay Buffer)	0.5 ml	1 ml	2 ml
HB3 (Blocking Buffer)	5 ml	10 ml	20 ml
HB4 (Capture Antibody, 200 µg/ml)*	7 µl	13 µl	26 µl
HB5 (Detection Antibody, 200 µg/ml)*	6 µl	10 µl	20 µl
HB6 (Developing Solution)	3 ml	6 ml	12 ml
HB7 (Stop Solution)	1.5 ml	3 ml	6 ml
HDAC2 Control (100 ng/µl)	8 µl	16 µl	32 µl
8-Well Assay Strip (with Frame)	3	6	12
User Guide	1	1	1

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

SHIPPING AND 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 redissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

Orbital shaker
Pipettes and pipette tips
Microplate reader
1.5 ml microcentrifuge tubes

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 10X HB1 with distilled water (pH 7.2-7.5) into 1X HB1.

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 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°C (without humidity) for 90 min 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 min.

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

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 min on a orbital shaker (50-100 rpm).

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

8. Dilute the 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 min.

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

10. Add 100 µl of HB6 to each well and incubate at room temperature for 2-10 min away from light. Monitor color development in the sample and standard well 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:

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

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 are omitted by mistake.
The well is not completely dried.	Ensure the well is incubated with no humidity and dry before adding block 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 is 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 positive control.
No Signal for Only the Sample	
The protein amount is added into the well insufficiently.	Ensure extract contains 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 isn't contaminated from adding the control protein or from using control protein contaminated tips.
Overdevelopment.	Decrease development time in step 10.