DATA SHEET

EpiQuik™ HDAC8 Assay Kit
Catalog No. P-4007

INTRODUCTION

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. HDAC8 is a class I histone deacetylase containing 377 amino acid residues. HDAC8 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 HDAC8 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™ HDAC8 Assay Kit addresses these problems by using a unique procedure to measure amount of HDAC8. The kit has following features:

• The fastest procedure, which can be finished within 3 hours.
• Innovative colorimetric assay to semi-quantitatively measure HDAC8 amount with no need of electrophoresis.
• Strip microplate format makes the assay flexible: manual or high throughput analysis.
• Simple, reliable, and consistent assay conditions. PRINCIPLE AND PROCEDURE The EpiQuik™ HDAC8 Assay Kit is designed for measuring total HDAC8 amount from tissues or cells. In an assay with this kit, the nuclear proteins containing HDAC8 are stably coated on the strip wells. The HDAC8 is recognized with highaffinity specific antibody. The amount of HDAC8 can be quantified through HRP conjugated secondary antibody-color development system and is proportional to the intensity of color development.

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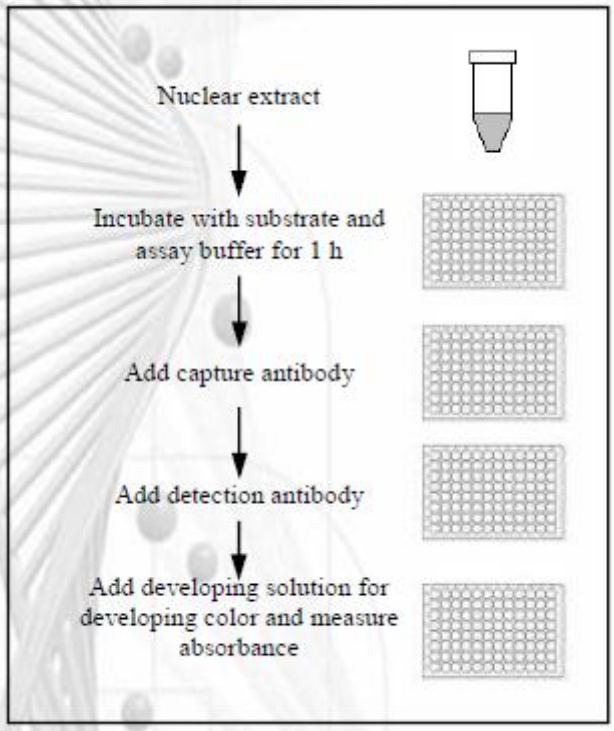

Schematic Procedure for Using the *EpiQuik*™ HDAC8 Assay Kit

PRODUCT USE INFORMATION

The EpiQuik™ HDAC8 Assay Kit is very suitable for measuring HDAC8 levels from various fresh tissues and cultured mammalian cells.
The EpiQuik™ HDAC8 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.
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EpiQuik™ is a trademark of Epigentek, Inc.

KIT CONTENTS

Components	24 assays P-4007-24	48 assays P-4007-48	96 assays P-4007-96
HC1 (10X Wash Buffer)	6 ml	11 ml	22 ml
HC2 (HDAC Assay Buffer)	0.5 ml	1 ml	2 ml
HC3 (Blocking Buffer)	5 ml	10 ml	20 ml
HC4 (Capture Antibody, 200 µg/ml)*	7 µl	13 µl	26 µl
HC5 (Detection Antibody, 200 µg/ml)*	6 µl	10 µl	20 µl
HC6 (Developing Solution)	3 ml	6 ml	12 ml
HC7 (Stop Solution)	1.5 ml	3 ml	6 ml
HDAC8 Control (100 ng/µl)	6 µl	12 µl	24 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: one part at ambient room temperature, and the second part on frozen ice packs at 4°C. Upon receipt: (1) Store HC5 and HDAC8 Control at –20°C; (2) Store HC1, HC3, HC4, HC6 and 8-Well Assay Strips at 4°C away from light; (3) Store HC2 and HC7 at room temperature. The kit is stable for up to 6 months from the shipment date, when stored properly.
Note: Check if wash buffer, HC1, 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 you own successful method. For your convenience and best results, Epigentek offers a nuclear extract kit (Cat# OP-0002-1) optimized for use in EpiQuikTM series. Nuclear extracts can be used immediately or stored at -800C for future use.

2. Determine number of the strip wells required (the strip wells can be broken off). Leave these strip wells in the plate frame (remaining unused strips can be put back in the bag. Seal the bag tightly and store at 4ºC). Dilute 10 X HC1 with distilled water (pH 7.2-7.5) to the 1 X HC1.

3. Adjust protein concentration to 0.4-1 µg/µl with HC2 and add 5 µl (2-5 µg) of the protein solution into 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 (with no humidity) for 60-90 min to evaporate the solution and completely dry the wells. For

the blank, add 5 µl of HC2 to the wells. For the positive control, dilute HDAC8 control to 2 30 ng/µl with HC2 and add 5 µl (10-150 ng) of diluted HDAC8 control solution to the wells.

4. Add 150 µl of HC3 to the dried wells and incubate at 37°C for 30-45 min

5. Aspirate and wash each well with 150 µl of 1 X HC1 three times.

6. Dilute the HC4 (at the 1:200 ratios) to 1 µg/ml with 1 X HC1. Add 50 µl of diluted HC4 to each assay strip well and 2 control strip wells. 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 1 X HC1 four times.

8. Dilute the HC5 (at the 1:1000 ratios) to 0.2 µg/ml with 1 X HC1. Add 50 µl of diluted HC5 to each strip well and incubate at room temperature for 30 min

9. Aspirate and wash each well with 150 µl of 1 X HC1 four times. Allow 3 min for last wash.

10. Add 100 µl of HC6 to each well and incubate at room temperature for 2-10 min away from light. Monitor color development in the sample and standard well (blue).

11. Add 50 µl of HC7 to each well and read absorbance on microplate reader at 450 nm.

12. Calculate HDAC8 level.

$$\text{HDAC8 level (OD/ml)} = (\text{sample OD} - \text{blank OD}) \times \text{sample dilution}$$

For accurate calculation, plot OD value versus amount of HDAC8 control and determine the slope as delta OD/ng. Calculate the amount of HDAC8 using the following formula:

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

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TROUBLESHOOTING

No Signal for Both the Positive Control and the Samples	
Reagents are added incorrectly.	Check if reagents are added in order and if some steps of 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 correctly followed.
No Signal or Very Weak Signal for Only the Positive Control	
The positive control is insufficiently added to the well.	Ensure sufficient amount of positive control is added.
The positive control 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 well insufficiently.	Ensure extract contains sufficient amount of proteins.
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 the protocol.
Contaminated by the positive control.	Ensure the well is not contaminated by adding positive control accidentally or by using positive control contaminated tips.
Overdevelopment.	Decrease development time in Step 10.