EpiQuik™ HAT Activity/Inhibition Assay Kit

Base Catalog # P-4003

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

The *EpiQuik*™ HAT Activity/Inhibition Assay Kit is very suitable for measuring HAT activity/inhibition from a broad range of species including mammalian cells/tissues, plants, and bacteria.

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KIT CONTENTS

Components	48 assays P-4003-48	96 assays P-4003-96
HT1 (10X Wash Buffer)	14 ml	28 ml
HT2 (HAT Substrate, 20 µg/ml)*	50 µl	100 µl
HT3 (HAT Assay Standard, 20 µg/ml)*	25 µl	50 µl
HT4 (Acetyl Co-A, 30 mM)*	10 µl	20 µl
HT5 (HAT Assay Buffer)	1.5 ml	3 ml
HT6 (Capture Antibody, 100 µg/ml)*	25 µl	50 µl
HT7 (Detection Antibody, 200 µg/ml)*	10 µl	20 µl
HT8 (Developing Solution)	6 ml	12 ml
HT9 (Stop Solution)	3 ml	6 ml
8-Well Assay Strip (with Frame)	6	12

** 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 HT2, HT3, HT4 and HT7 at –20°C away from light; (2) Store HT1, HT6, HT8, and 8-Well Assay Strips at 4°C away from light; (3) Store all remaining components at room temperature. All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Note: Check if wash buffer, HT1, 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*™ HAT Activity/Inhibition Assay Kit is for research use only and is not intended for diagnostic or therapeutic application.

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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. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Intellectual Property: *EpiQuik*™ is a trademark of EpigenTek, Inc.

A BRIEF OVERVIEW

Histone acetylases (HATs) play a critical role in transcriptional activation of gene expression in eukaryotic cells through modifying N-terminal lysine residues of histones by the addition of an acetyl group from acetyl coenzyme A. More than 20 HATs have been identified and these HATs can be classified into five families: GNAT1, MYST, TAFII250, P300/CBP, and nuclear receptor co-activators such as ACTR. HAT activation or inhibition displays significant effects on several diseases ranging from neurodegenerative disorders to cancer. The impact of HATs on cellular physiology and disease would benefit from the identification of specific pharmacological inhibitors. Several HAT inhibitors are developed to date. There are several methods used for measuring HAT activity/inhibition. However, most of these methods available so far are to indirectly measure HAT activity through detecting generation of free CoA or CoA-SH, which may cause the measured HAT activity and inhibitory effects of inhibitors to be less accurate.

The *EpiQuik*™ HAT Activity/Inhibition Assay Kit uses a proprietary and unique procedure to measure HAT activity/inhibition with the following features:

- Fast procedure, which can be finished within 3 hours.
- Innovative colorimetric assay without the use of radioactivity, extraction, or chromatography.
- Direct measurement of HAT activity and inhibition by quantifying the amount of acetylated histone substrate, thereby avoiding the false inhibitory effect on HATs.
- Strip microplate format makes the assay flexible: manual or high throughput analysis.
- Simple, reliable, and consistent assay conditions.

PRINCIPLE & PROCEDURE

The *EpiQuik*™ HAT Activity/Inhibition Assay Kit is designed for measuring total HAT activity/inhibition. In an assay with this kit, the unique histone substrate is stably captured on the strip wells. Active HATs bind to and acetylate histone substrate. The acetylated substrate can be recognized with a high affinity anti-acetylated histone antibody. The ratio or amount of the acetylated histone, which is directly proportional to HAT enzyme activity, can then be colorimetrically quantified through an ELISA-like reaction.

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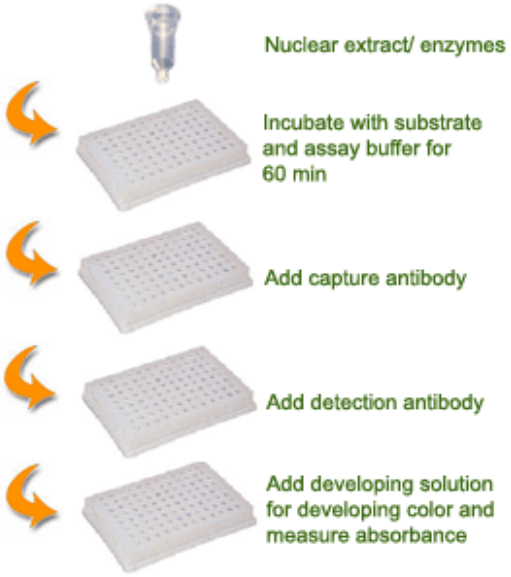

Schematic Procedure for Using the *EpiQuik*™ HAT Activity/Inhibition Assay Kit

PROTOCOL

1. Prepare nuclear extracts by using you 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. 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°C). Dilute **HT1** with distilled water (pH 7.2 to 7.5) at a 1:10 ratio (ex: 1 ml of **HT1** + 9 ml of distilled water).

3. Dilute **HT2** at a 1:50 ratio with **diluted HT1**, and then add 50 µl of the **diluted HT2** into each well, except the wells for the standard curve. For preparation of the standard curve, add 50 µl of **diluted HT1** into the wells (no **HT2**), followed by adding 1 µl of **HT3** at different amounts (0.1 – 10 ng); **HT3** can be diluted with **diluted HT1** to achieve your different concentration points. Cover the wells with Parafilm M and incubate at room temperature for 30-45 minutes.

4. Aspirate and wash each well with 150 µl of **diluted HT1** three times.

5. Dilute **HT4** at a 1:20 ratio with **HT5**. Add 26 µl of **HT5**, 2 µl of the **diluted HT4**, and 2 µl of nuclear extracts (4-20 µg) or HAT enzymes to each strip well. Mix, cover the strip wells, and incubate at 37°C for 30-60 minutes. For the standard curve, add 2 µl of **HT5** instead of nuclear extracts or HAT enzymes. For HAT inhibition, add 2 µl of different amounts of the tested inhibitors and reduce **HT5** volume to 24 µl. For the blank, add 30 µl of **HT5** into the blank wells.

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6. Aspirate and wash each well with 150 µl of **diluted HT1** three times.

7. Dilute **HT6** (at a 1:100 ratio) to 1 µg/ml with **diluted HT1**. Add 50 µl of the **diluted HT6** to each strip well and incubate at room temperature for 60 minutes on an orbital shaker (50-100 rpm).

8. Aspirate and wash each well with 150 µl of **diluted HT1** four times.

9. Dilute **HT7** (at a 1:1000 ratio) to 0.2 µg/ml with **diluted HT1**. Add 50 µl of the **diluted HT7** to each strip well and incubate at room temperature for 25-30 minutes.

10. Aspirate and wash each well with 150 µl of **diluted HT1** four to five times.

11. Add 100 µl of **HT8** 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).

12. Add 50 µl of **HT9** to each well to stop enzyme reaction when the color in the standard wells containing the higher concentrations of standard control turns medium blue. The color should change to yellow and absorbance can be read on a microplate reader at 450 nm within 2-15 minutes.

13. Calculate HAT activity or inhibition. For simple calculation:

$$\text{HAT activity (OD/h/mg protein)} = \frac{\text{OD (untreated sample – blank)}}{\text{hour x protein amount (µg) added into the assay}} \times 1000$$

$$\text{Inhibition \%} = \left(1 - \frac{\text{OD (inhibitor sample – blank)}}{\text{OD (no inhibitor control – blank)}}\right) \times 100\ \%$$

For an accurate calculation, plot OD value versus amount of **HT3** and determine the slope as delta OD/ng.

Calculate HAT activity using the following formula:

$$\text{Activity (ng/h/mg)} = \frac{\text{OD (untreated sample – blank)}}{\text{slope} \times \text{hour} \times \text{protein amount (µg) added into the assay}} \times 1000$$

TROUBLESHOOTING

No Signal for the Sample

The protein sample is not properly extracted.	Ensure the protein extraction protocol is suitable for nuclear protein extraction.

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The protein amount is added into well insufficiently.	Ensure extract contains a sufficient amount of protein.
The sample is not prepared from fresh cells or tissues.	The nuclear extracts from frozen cells or tissues significantly lose enzyme activity. A fresh sample should be used.
Nuclear extracts are incorrectly stored.	Ensure the nuclear extracts are stored at –80°C.
Reagents are added incorrectly.	Check if reagents are added in order and if any steps of the procedure 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.
Absence of HAT activity in the sample due to treatment.	N/A.

High Background Present for the Blank

The well is not washed sufficiently.	Check if wash at each step is performed according to the protocol.
Overdevelopment.	Decrease development time in step 11.

RELATED PRODUCTS

P-4002	*EpiQuik*™ HDAC Activity/Inhibition Assay Kit (Colorimetric)
P-4005	*EpiQuik*™ HDAC1 Assay Kit
P-4006	*EpiQuik*™ HDAC2 Assay Kit
P-4007	*EpiQuik*™ HDAC8 Assay Kit

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