KAMIYA BIOMEDICAL COMPANY

HAT Activity Assay

For the rapid and sensitive colorimetric measurement of HAT activity in mammalian samples

Cat. No. KT-146

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** HAT Activity Assay is for the rapid and sensitive colorimetric measurement of HAT activity in mammalian samples. For research use only, not for use in diagnostic procedures.

PRINCIPLE
Histone acetyltransferases (HATs) have been implicated to play a crucial role in a variety of cellular functions, such as gene transcription, differentiation, and proliferation. The kit utilizes active Nuclear Extract (NE) as a positive control and acetyl-CoA as a cofactor. Acetylation of peptide substrate by active HAT releases the free form of CoA, which then serves as an essential co-enzyme for producing NADH. NADH can easily be detected spectrophotometrically upon reacting with a soluble tetrazolium dye. Unlike conventional radioisotope methods, the assay continuously measures HAT activity and thus is suitable for kinetic studies. Since histone deacetylases do not interfere with this assay, crude nuclear extracts may be used directly. The kit provides a simple, straightforward protocol for a complete assay.

COMPONENTS

• HAT Assay Buffer (2X)	7.5 mL	(Amber cap)
• HAT Substrate I	1 vial	(Blue cap)
• HAT Substrate II	1 vial	(Purple cap)
• NADH Generating Enzyme	800 µL	(Green cap)
• Nuclear Extract (NE), 4 µg/µL	50 µL	(Red cap)
• HAT Reconstitution Buffer	1.8 mL	(Clear cap)

PROTOCOLS
A. Reagent Preparations:
- Reconstitute HAT Substrate I, HAT Substrate II with 550 µL HAT Reconstitution Buffer each. The Substrate II will be cloudy, and milky-brown. Pipette up and down several times to dissolve. The reagents are stable for two months at -80°C after reconstitution.
- Nuclear Extract or purified protein samples may be tested using this kit.
- Use of U-shaped 96-well plates may increase signal up to 40% in comparison to the flat-shaped plates.

B. HAT Assay Protocol:
1. Prepare test samples (50 µg of nuclear extract or purified protein) in 40 µL water (final volume) for each assay in a 96-well plate. For background reading, add 40 µL water instead of sample. For positive control, add 10 µL of the NE (Cell Nuclear Extract) and 30 µL water.
2. Assay Mix preparation: Mix enough reagents for the number of assays performed. For each well, prepare a total of 65 µL Assay Mix containing:
   a. 50 µL of HAT Assay Buffer (2X)
   b. 5 µL of HAT Substrate I
   c. 5 µL of HAT Substrate II (mix before use)
   d. 8 µL of NADH Generating Enzyme
3. Mix the prepared Assay Mix, add 68 µL of Assay Mix to each well, mix to start the reaction.

4. Incubate plates at 37°C for 1 – 4 hours, depending on the color development. Read samples in a plate reader at 440 nm. For kinetic studies, read O.D. 440 nm at different times during the incubation.

   Notes:
   a. The yellow color develops slowly, but very steadily, and is repeatable.
   b. Background reading from buffer and reagents (without HAT) is significant, which should be subtracted from the readings of all samples.
   c. HAT activity can be expressed as the relative O.D. value per µg or nmol/min/µg
      Sample: $\varepsilon_{440\ nm} = 37{,}000\ M^{-1}cm^{-1}$ under the assay conditions.

STORAGE

Store at -80°C until the expiration date.

GENERAL PRECAUTION

- Samples containing DTT, Co-enzyme A, and NADH should be avoided, as these compounds strongly interfere with the reactions.

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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