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Product Information

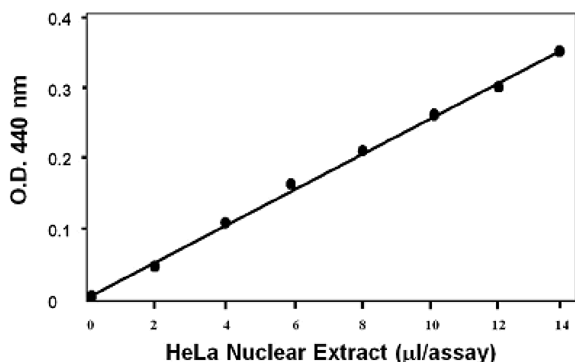
HAT Activity Colorimetric Assay Kit

Catalog Number EPI001
Storage Temperature – 20 °C

TECHNICAL BULLETIN

Product Description
Histone acetyltransferases (HATs) have critical roles in cellular functions and are involved in the regulation of gene transcription, differentiation, and proliferation. The HAT Activity Colorimetric Assay Kit offers a convenient, non-radioactive system for a rapid and sensitive detection of HAT activity in mammalian samples. The kit includes an active Nuclear Extract (NE) to be used as a positive control, HAT cofactor, acetyl-CoA, and all downstream cofactors and substrates. Acetylation of a peptide substrate by active HAT releases the free form of CoA, which then serves as an essential coenzyme for producing NADH. NADH can easily be detected spectrophotometrically upon reacting with a soluble tetrazolium dye.

The kit provides all reagents and a simple, straightforward protocol to assay HAT activity – just combine nuclear extract or protein sample with reagents, incubate, and read absorbance. Unlike the conventional radioisotope method, this colorimetric assay continuously measures HAT activity, and therefore, is suitable for kinetic studies. In addition, histone deacetylases do not interfere with the assay, and therefore, crude nuclear extract can be used directly in the assay.



Analyses of HAT Activity in HeLa Nuclear Extract.
A dilution series of HeLa nuclear extract was incubated with HAT Assay Mix according to the kit instructions. Activity was measured in a micro plate reader at 440 nm and plotted to evaluate linear response.

Components
The kit is sufficient for 100 assays in 96 well plates.

Component	Amount
2X HAT Buffer (Amber cap) Catalog Number EPI001A	7.5 ml
HAT Substrate I (Blue cap) Catalog Number EPI001B	1 vl
HAT Substrate II (Purple cap) Catalog Number EPI001C	1 vl
NADH Generating Enzyme (Green cap) Catalog Number EPI001D	800 µL
Nuclear Extract (NE, 4 mg/ml) (Red cap) Catalog Number EPI001E	50 µL
HAT Reconstitution Buffer(Clear cap) Catalog Number EPI001F	1.8 ml

Reagents and equipment required but not provided.
96 well U--shaped plate. Use of U-shaped 96-well plates is recommended, and may increase signal up to 40% in comparison to flat bottom plate

Microplate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on wet ice. All other components should be stored at –20 °C, protected from light.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Read the entire protocol before performing the assay.

Procedure

All samples and standards should be run in duplicate.

1. Reagent Preparation and General Precautions

Reconstitute both HAT Substrate I and Substrate II with 550 µL HAT Reconstitution Buffer. Reconstituted Substrate II will be become cloudy and a milky-brown color. 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 can be tested using this kit

Note: Reagents containing DTT, Coenzyme A, and NADH should be avoided in preparing nuclear extracts, as these compounds strongly interfere with the assay. Native or pre-existing levels in nuclei will not interfere.

2. Sample Preparation

For each test assay

a. Prepare test samples by diluting 50 µg of nuclear extract or purified protein based on protein assays such as BCA/Bradford assay in water to a final volume of 40 µL per well in a 96-well plate.
b. For background reading, prepare a well with 40 µL water alone
c. For a positive control, combine 10 µL of the Nuclear Extract (NE) and 30 µl water.

3. Assay Mix preparation

a. Prepare sufficient Assay Mix for the number of assays to be performed. For each assay, prepare 68 µL assay mix containing:

Reagent	Volume
2X HAT Assay Buffer	50 µL
HAT Substrate I	5 µL
HAT Substrate II (Mix before use)	5 µL
NADH Generating Enzyme	8 µL
Total volume	68 µL

Mix thoroughly

Note: Assay mix and test samples must be at room temperature before starting the assay.

b. add 68 µL to each well, and mix by gently pipetting up and down to start the reaction

4. Incubation, Absorbance Readings

Incubate plates at 37°C for 1 ~ 4 hours depending level of HAT activity and therefore, rate of the color development

Read sample in a plate reader at 440 nm. For kinetic studies, read O.D. 440nm at frequent time intervals during incubation.

Notes

a. The yellow color develops slowly, but very steadily and reproducibly.
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. ε_{440nm} = 37000 $M^{-1}cm^{-1}$ under the kit assay conditions.

Troubleshooting Guide

Problems	**Cause**	**Solution**
Assay not working	Use of ice-cold Assay Mix	Assay Mix must be at room temperature
	Omission of a step in the protocol	Refer and follow the data sheet precisely
	Plate read at incorrect wavelength	The filter setting of the instrument should be 440 nm
	Use of an incompatible 96-well plate	Use 96 well Clear plates
Samples with erratic readings	Use of an incompatible sample type	Avoid samples with DTT, coenzyme A and NADH
	Samples prepared in an incompatible buffer	Use the assay buffer provided in the kit
	Samples used after multiple free-thaw cycles	Aliquot and freeze samples if needed for multiple assays. Avoid multiple freeze-thaw cycles.
	Use of old or inappropriately stored samples	Use fresh samples or store at correct temperatures until use
Lower/Higher readings in Samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Always check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix immediately before each use
	Incorrect incubation times or temperatures	Refer to procedure and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in the standard	Avoid pipetting small volumes
	Pipetting errors in the reaction mix	Prepare a master reaction mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the wells
	Substituting reagents from older kits/ lots	Use fresh components from the same kit
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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