HAT Activity Colorimetric Assay Kit

(Catalog # K332-100; 100 assays; Store at -80°C)

I. Introduction:

Histone acetyltransferases (HATs) have been implicated in a crucial role in various cellular functions, such as gene transcription, differentiation, and proliferation. BioVision's HAT Activity Colorimetric Assay Kit offers a convenient, nonradioactive system for a rapid and sensitive detection of HAT activity in mammalian samples. 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 coenzyme for producing NADH. NADH can easily be detected spectrophotometrically upon reacting with a soluble tetrazolium dye. The detection can be continuous and suitable for kinetic studies. The kit provides a simple, straightforward protocol for a complete assay.

II. Kit Contents:

Component	K332-100 100 assays	Cap Color	Part Number
2X HAT Buffer	7.5 ml	Amber	K332-100-1
HAT Substrate I	1 vial	Blue	K332-100-2
HAT Substrate II	1 vial	Red	K332-100-3
NADH Generating Enzyme	800 µl	Green	K332-100-4
Nuclear Extract (NE, 4 mg/ml)	50 µl	Violet	K332-100-5
HAT Reconstitution Buffer	1.8 ml	Clear	K332-100-6

III. Reagent Preparation and General Precaution:

- Reconstitute HAT Substrate I, substrate II with 550 µl HAT Reconstitution Buffer. The Substrate II will be become brown cloudy and milky 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. **For the nuclear extract preparation, please refer to the *Nuclear/Cytosol Fractionation Kit* (BioVision, K266-100) without using DTT, as DTT interferes with the assay.**
- Samples containing DTT, Coenzyme A, and NADH should be avoided, as these compounds strongly interfere with the reactions.
- Using U-shaped 96-well plates may increase signal up to 40 % in comparison to flat bottom plates.

IV. 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 68 µl Assay Mix containing:

   50 µl 2X HAT Assay Buffer
   5 µl HAT Substrate I
   5 µl HAT Substrate II (Mix before use)
   8 µl 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 hr depending on the color development. Read sample in a plate reader at 440 nm. For kinetic studies, read O.D. 440 nm at different times during incubation.

Notes:

1) The yellow color develops slowly, but very steadily and repeatable.
2) Background reading from buffer and reagents (without HAT) is significant, which should be subtracted from the readings of all samples.
3) 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.

Advantages: The HAT Activity Colorimetric Assay (K332-100) provides an easy and very simple procedure to assay HAT activity (just adding reagents to sample preparations incubate and read). Unlike the conventional radioisotope method, the assay continuously measures HAT activity and thus is suitable for kinetic studies. In addition, the assay is not interfered by the presence of histone deacetylases and therefore, crude nuclear extract can be used directly in the assay.

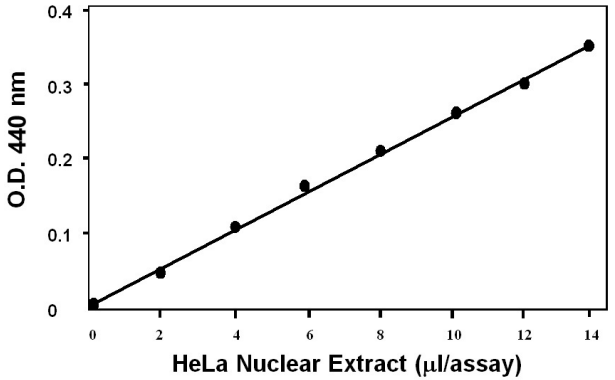

Fig. 1. Analyses of HAT Activity in HeLa Nuclear Extract. HeLa nuclear extract (Cat.# 1641-1) in various amounts was incubated with HAT substrate. Activity was analyzed in a micro plate reader at 440 nm according to the kit instructions.

RELATED PRODUCTS:

- Cell Fractionation System
- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Cytosol/Particulate Rapid Separation Kit
- FractionPREP Fractionation System
- Cell Proliferation & Senescence Assays
- Cell Damage & Repair
- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- DNA Damage Quantification Kit and more

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• 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 expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & 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 tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		