

# HAT Activity Colorimetric Assay Kit

(Catalog #ALX-850-326; 100 assays; Store at  $-80^{\circ}\text{C}$ )

## I. Introduction:

Histone acetyltransferases (HATs) have been implicated in a crucial role in various cellular functions, such as gene transcription, differentiation, and proliferation. The Enzo Life Sciences (ELS) 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	ALX-850-326	Cap Color	Part Number
	100 assays		
2X HAT Buffer	7.5 ml	Amber	ALX-K332-100-1
HAT Substrate I	1 vial	Blue	ALX-K332-100-2
HAT Substrate II	1 vial	Red	ALX-K332-100-3
NADH Generating Enzyme	800 $\mu\text{l}$	Green	ALX-K332-100-4
Nuclear Extract (NE, 4 mg/ml)	50 $\mu\text{l}$	Purple	ALX-K332-100-5
HAT Reconstitution Buffer	1.8 ml	Clear	ALX-K332-100-6

## III. Reagent Preparation and General Precaution:

- Reconstitute HAT Substrate I, substrate II with 550  $\mu\text{l}$  HAT Reconstitution Buffer. The Substrate II will become brown cloudy and milky color. Pipette up and down several times to dissolve. The reagents are stable for two months at  $-80^{\circ}\text{C}$  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 (BV-K266, [www.axxora.com](http://www.axxora.com)) 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:

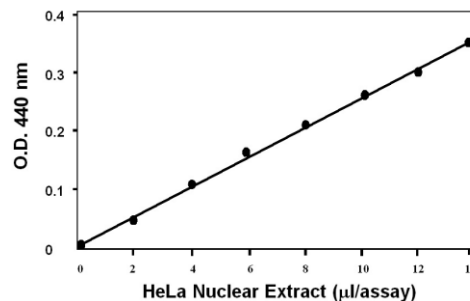
- Prepare test samples (50  $\mu\text{g}$  of nuclear extract or purified protein) in 40  $\mu\text{l}$  water (final volume) for each assay in a 96-well plate. For background reading, add 40  $\mu\text{l}$  water instead of sample. For positive control, add 10  $\mu\text{l}$  of the NE (Cell Nuclear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

- Incubate plates at  $37^{\circ}\text{C}$  for 1 ~ 4 hours depending on the color development. Read sample in a plate reader at 440 nm. For kinetic studies, read O.D. 440nm at different times during incubation.

## Notes:

- The yellow color develops slowly, but very steadily and repeatable.
- Background reading from buffer and reagents (without HAT) is significant, which should be subtracted from the readings of all samples.
- HAT activity can be expressed as the relative O.D. value per  $\mu\text{g}$  or  $\text{nmol}/\text{min}/\mu\text{g}$  sample.  $\epsilon_{440\text{nm}} = 37000 \text{ M}^{-1}\text{cm}^{-1}$  under the kit assay conditions.

**Advantages:** The HAT Activity Colorimetric Assay (ALX-850-326) 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.

## IV. 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

**GENERAL TROUBLESHOOTING GUIDE:**

<b>Problems</b>	<b>Cause</b>	<b>Solution</b>
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed</li> <li>• Use fresh samples or store at correct temperatures until use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>
<b>Note#</b> The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		