Introduction and Background

Histone acetyltransferases (HATs) have been implicated to play a crucial role in various cellular functions, such as gene transcription, differentiation, and proliferation. The HAT Activity 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.

Material and Method

A. List of component

1. 2X HAT Assay Buffer: 7.5 ml.
2. HAT Substrate I: 1 vial.
3. HAT Substrate II: 1 vial.
4. NADH Generating Enzyme: 1 vial.
5. NE (Nuclear Extract 4 mg/ml): 50 µl.
6. HAT Reconstitution Buffer: 1.8 ml.

B. Reagent Preparations and General Precaution

- Reconstitute HAT Substrate I, substrate II, and NADH Generating Enzyme each with 550µl 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 month at -80℃ after reconstitution.
- Nuclear Extract or purified protein samples can be tested using this kit. For nuclear extract preparation, 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.
- Use U-shape 96-well plates may increase signal up to 40% in comparison to the flat shape plates.

C. 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 30µl water.
2. Assay Mix preparation: Mix enough reagents for the number of assays performed. For each well, prepare a total 65 µl Assay Mix containing: 50 µl of 2X HAT Assay Buffer, 5 µl HAT Substrate I, 5 µl HAT Substrate II (Mix before use), 5 µl NADH Generating Enzyme.

3. Mix the prepared Assay Mix, add 65 µl of Assay Mix to each well, mix to start the reaction.
4. Incubate plates at 37℃ for 1-4 hours 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 steady 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 Kit 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.

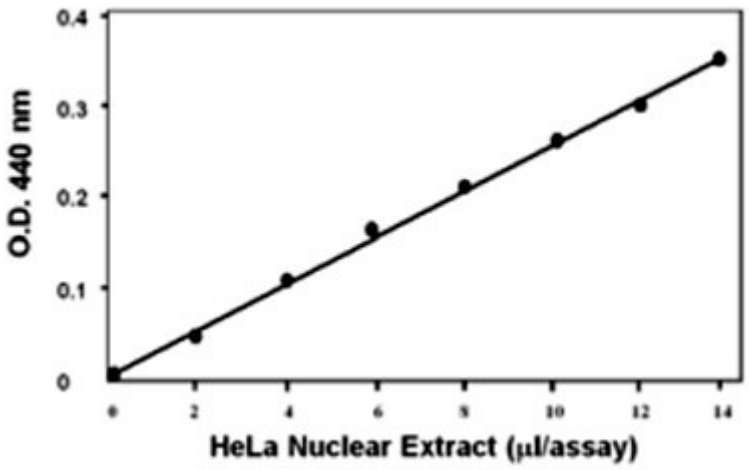

Fig. 1. Analyses of HAT Activity in HeLa Nuclear Extract. HeLa nuclear extract in various amounts was incubated with HAT substrate. Activity was analyzed in a micro plate reader at 440 nm according to the kit instructions.