Product Information

HAT Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2033-100 100 assays	Cap Color	Part Number
2X HAT Buffer	7.5 ml	Amber	K2033-C-1
HAT Substrate I	1 vial	Blue	K2033-C-2
HAT Substrate II	1 vial	Purple	K2033-C-3
NADH Generating Enzyme	800 µl	Green	K2033-C-4
Nuclear Extract (NE, 4 mg/ml)	50 µl	Red	K2033-C-5
HAT Reconstitution Buffer	1.8 ml	Clear	K2033-C-6

II. Introduction:

Histone acetyltransferase (HAT) is an enzyme that acetylates conserved lysine on a histone protein by transferring an acetyl group from acetyl CoA. Histone acetylation can increase gene expression. HAT plays important roles in gene transcription, cell proliferation and differentiation.

The HAT Activity Colorimetric Assay Kit provides a fast and sensitive way for detection of HAT activity in mammalian samples based on colorimetric method that eliminates radioactivity in traditional assays. The assay utilizes active Nuclear Extract (NE) as a positive control and acetyl-CoA as a cofactor. The active HAT acetylates peptide substrate and then releases the free form of CoA which then acts as an essential coenzyme for generating NADH. NADH can be easily detected upon reacting with a soluble tetrazolium dye using a spectrophotometer or an ELISA plate reader. The detection can be continuous and suited for kinetic studies.

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℃ 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 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:

2X HAT Assay Buffer	50 µl
HAT Substrate I	5 µl
HAT Substrate II (Mix before use)	5 µl
NADH Generating Enzyme	8 µl

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℃ 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.

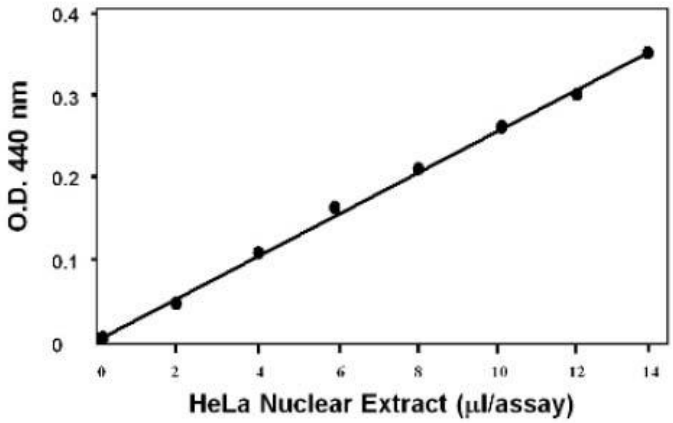
Notes:

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. E_{440nm} = 37000 $M^{-1}cm^{-1}$ under the kit assay conditions.

Advantages: The HAT Activity Colorimetric Assay 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.



General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the Nucleotide releasing buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately

and Standards	• Incorrect incubation times or temperatures • Incorrect volumes used	• Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

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Our promise

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Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com