New Positive Control Storage Temperature

Acetyltransferase activity kit

Catalog # **ADI-907-026**

Sufficient Reagents for 96 tests
For use with purified in vitro samples

Reagents require separate storage conditions.

Check our website for additional protocols, technical notes and FAQs.

For proper performance, use the insert provided with each individual kit received.

Table of Contents

Patent Pending

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

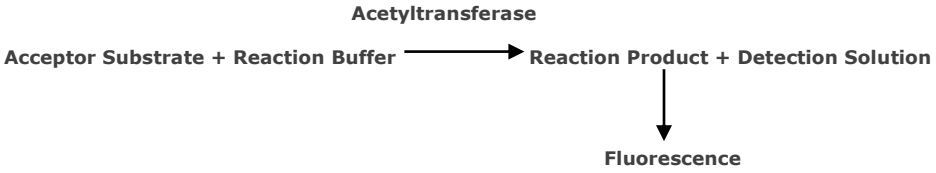
Introduction

The Acetyltransferase activity kit is a complete kit for the screening of candidate compounds that may alter normal acetyltransferase activity. Please read the complete kit insert before performing this assay.

Acetylation is an important covalent molecular modification. Originally identified as the method by which certain bacteria were able to deactivate anti-microbial compounds, acetylation is now also known as an important partitioning and signaling modification.[1] Acetyltransferases are enzymes that covalently transfer an acetyl group from a donor molecule (Acetyl CoA) to an acceptor. Acetyl CoA serves as a universal donor while the acceptor varies with the acetyltransferase. Acceptors include histones, kinases, transcription factors, receptors, neurotransmitter precursors like choline and serotonin, and anti-microbial agents like chloramphenicol and fluoroquinones.[2-6] Acetylation can signal an increase or decrease in activity based on the context of the message.[7] Frequently located at critical junctions in metabolic pathways, Acetyltransferases and their regulation have become attractive therapeutic targets to treat everything from insomnia to cancer.[8-10]

Principle

1. Enzyme and reaction mix spiked with acceptor substrate are added to wells of a black 96-well plate. The plate is then incubated.
2. Ice cold isopropyl alcohol is added to stop the reaction. The detection solution is then added and the plate is again incubated.
3. The plate is transferred to a plate reader and fluorescence is measured at 380ex/520em.



Materials Supplied

Do not mix components from different kit lots or use reagents beyond the kit's expiration date.

1. **Black Microtiter Plate**
   One plate of 96 wells, Catalog No. 80-1675
   The plate is ready to use.

2. **Transferase Assay Buffer Concentrate**
   15 mL, Catalog No. 80-1648
   A 10X concentrated buffer containing detergent and preservative.

3. **Acetyltransferase Reaction Buffer Concentrate**
   0.1 mL, Catalog No. 80-1651
   A 50X concentrated buffer containing Acetyl CoA.

4. **Transferase Detection Solution Concentrate.**
   0.15 mL, Catalog No. 80-1650
   A 100X concentrated solution of fluorescent substrate in DMSO.

5. **Acetyltransferase Positive Control**
   0.2 mL, Catalog No. 80-1647
   A Stock Solution of buffer and reaction end product.

Detection Solution contains DMSO. Avoid skin contact. Avoid breathing vapor.

6. **Foil Plate Sealer**
   3 each, Catalog No. 10-3126

7. **Acetyltransferase Assay Layout Sheet**
   1 each, Catalog No. 30-0240

Storage

Reagents require separate storage conditions.

Acetyltransferase Reaction Buffer Concentrate and Transferase Detection Solution concentrate **must** be stored at -20°C, and Acetyltransferase Positive Control **must** be stored at -70°C. Transferase Assay buffer Concentrate can be stored at -20°C or 4°C. All kit components are stable at their recommended storage temperatures until the kit expiration date. Recommended storage temperatures do not necessarily reflect shipping conditions.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Acetyl CoA dependent acetyltransferase.
3. Appropriate acceptor substrate.
4. Inhibitor/activator compounds to be screened.
5. Precision pipets for volumes between 5 µL and 1,000 µL.
6. Disposable beakers for diluting buffer concentrates.
7. Graduated cylinders.
8. A microplate shaker.
9. Disposable microtubes, 0.5 and 1.5 mL.
10. Microplate reader capable of measuring fluorescence at 380ex/520em.
11. Crushed ice and container.
12. Isopropyl alcohol (ice cold).

Reagent Preparation

Thaw Detection Solution Concentrate at room temperature. Do not place on ice. Thaw all other reagents on ice.

1 Transferase Assay Buffer

Prepare the assay buffer by diluting 15mL of the supplied Transferase Assay Buffer Concentrate with 135mL of deionized water. This solution can be stored at 4°C for 3 months, or the kit's expiration, whichever is earlier. The 1X assay buffer is used to prepare dilutions of Acetyltransferase Reaction Buffer, Transferase Detection Solution, enzymes, substrates, and compounds to be screened.

Pre-rinse each pipet tip with reagent.

2 Transferase Detection Solution

Count the total number of wells needed for compound screening and add 6 (for the zero, positive control, and blank wells in duplicate). Use the following formula to calculate the volume of 1X Detection Solution required.

Store 1X Detection Solution on ice and use within 4 hours of preparation. Discard unused 1X Solution.

A. Total volume required

   [Total number of wells needed + 6] x 100 µL = _____ µL

B. Volume of Transferase Detection Solution Concentrate required

   [Total volume required (from A. above)] x 0.01 = _____ µL

C. Volume of 1X Transferase Assay Buffer required

   [Total volume required (from A. above)] x 0.99 = _____ µL

A minimum of 2 wells of the positive control are recommended. Adjust the calculations accordingly if more than 2 wells are used

Prepare 1X Detection Solution by combining the appropriate reagent volumes calculated in B and C above. For example, to prepare 2 mL of 1X Detection Solution, combine the following volumes: 20 µL of the supplied Transferase Detection Solution Concentrate and 1980 µL 1X Transferase Assay Buffer. Diluted Detection Solution should be kept on ice and used within 4 hours of preparation. Any unused 1X Detection Solution should be discarded.

Detection Solution contains DMSO. Avoid skin contact. Avoid breathing vapor.

3 Acetyltransferase Reaction Mix

Count the total number of wells needed for compound screening and add 4 (for the zero and blank wells, in duplicate). Use the following formula to calculate the volume of 1X Acetyltransferase Reaction Mix required.

A. Total volume required

   [Total number of wells needed + 4] x 25 µL = _____ µL

B. Volume of Acetyltransferase Reaction Buffer Concentrate required

   [Total volume required (from A. above)] x 0.02 = _____ µL

C. Volume of acceptor substrate required

   [Determined empirically based on enzyme used] = _____ µL

D. Volume of 1X Transferase Assay Buffer required

[Total volume required (from A. above)] x 0.98 – [volume of substrate required] = _____ µL

Prepare 1X Reaction Mix **on ice** by combining the appropriate reagent volumes calculated in B, C and D above. For example, to prepare 2 mL of Reaction Mix spiked with 22 µL of acceptor substrate, combine the following volumes: 40 µL Acetyltransferase Reaction Buffer Concentrate, 22 µL substrate and 1938 µL 1X Transferase Assay Buffer. Diluted Reaction Mix should be kept on ice and used within 8 hours of preparation. Any unused 1X Reaction Mix should be discarded.

4 Positive Control

Thaw Acetyltransferase Positive Control on ice.

Store Diluted Positive Control on ice and use within 2 hours of preparation. Discard unused 1X solution.

A positive control is included in the kit to verify the activity of the kit components. It should not be used to calculate the concentration of acetyltransferase activity in samples. Store the Positive Control at -70°C and avoid freeze-thaws.

Prepare enough Positive Control to test in duplicate. Use the following formula to calculate the volume of Positive Control required. A minimum of 2 wells of the positive control are recommended per assay.

A. Total volume required

[Total number of wells needed] x 50µL = _____ µL

B. Dilute Acetyltransferase Positive Control Stock 1:100 by adding 5µL of Positive Control to 495µL of 1X Transferase Assay Buffer.

C. Volume of 1:100 Positive Control required

[Total volume required (from A. above)] x 0.025 = _____ µL

D. Volume of 1X Transferase Assay Buffer required

[Total volume required (from A. above)] x 0.975 =_____ µL

Prepare Positive Control **on ice** by combining the appropriate reagent volumes calculated in C and D above. For example, to prepare 200µL of Positive Control, combine the following volumes: 5µL of (1:100) Positive Control and 195µL of 1X Transferase Assay Buffer. Diluted Positive Control should be kept on ice and used within 2 hours of preparation. Any unused 1X Positive Control should be discarded.

Sample Handling

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

This assay is suitable for use with all Acetyl CoA-dependent acetyltransferases. It is necessary to titrate each enzyme / substrate system in the assay to determine optimal conditions.

This assay should only be used to screen purified *in vitro* samples in buffer systems without reductants.

Before screening colored compounds, the compound should be titered in the assay to ensure that there is no interference with signal collection.

It is recommended that an end-point assay be performed to determine the optimal concentration of enzyme/substrate to use prior to screening candidate compounds. Make serial dilutions of the acetyltransferase of interest in the assay buffer. Initial concentrations of 100 nM are recommended. A kinetic assay format is also an available option.

The positive control provided may also be used to test colored compounds for interference in the assay. A suitable protocol follows.

End point / Kinetic Assay Procedure

All samples should be run in duplicate.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Cover unused wells tightly with a plate sealer. **DO NOT REUSE WELLS!**

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample and reagent.

1. Pipet 25 µL of 1X Transferase Assay Buffer into the blank wells.
2. Pipet 25 µL of acetyltransferase dilutions into the bottom of the appropriate wells.
3. Pipet 25 µL of 1X Reaction Mix into each well.
4. Cover plate with foil plate sealer. Incubate for 30 min (for end point format) shaking* at room temperature.

   For **Kinetic** measurements, incubate identical reaction wells for the desired periods of time. The duplicate wells for each time point can be stopped as in step #6 below.
5. Pipet 50 µL of 1X Positive Control into the bottom of the appropriate wells.
6. Pipet 50 µL of ice cold isopropyl alcohol into each well.
7. Pipet 100 µL 1X Detection Solution into each well.
8. Cover plate with foil plate sealer. Incubate for 10 min at room temperature, without shaking.
9. Read fluorescence at 380ex/520em.

Pipet the reagents to the sides of the wells to avoid possible contamination.

* Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.

Example of Endpoint Assay Results

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

1. Plot the mean of the duplicate relative fluorescence units (RFU) at 380ex/520em versus Enzyme concentration. (Figure 1)
2. Calculate the signal-to-noise ratio : Mean RFU for enzyme dilution / Mean RFU blank.

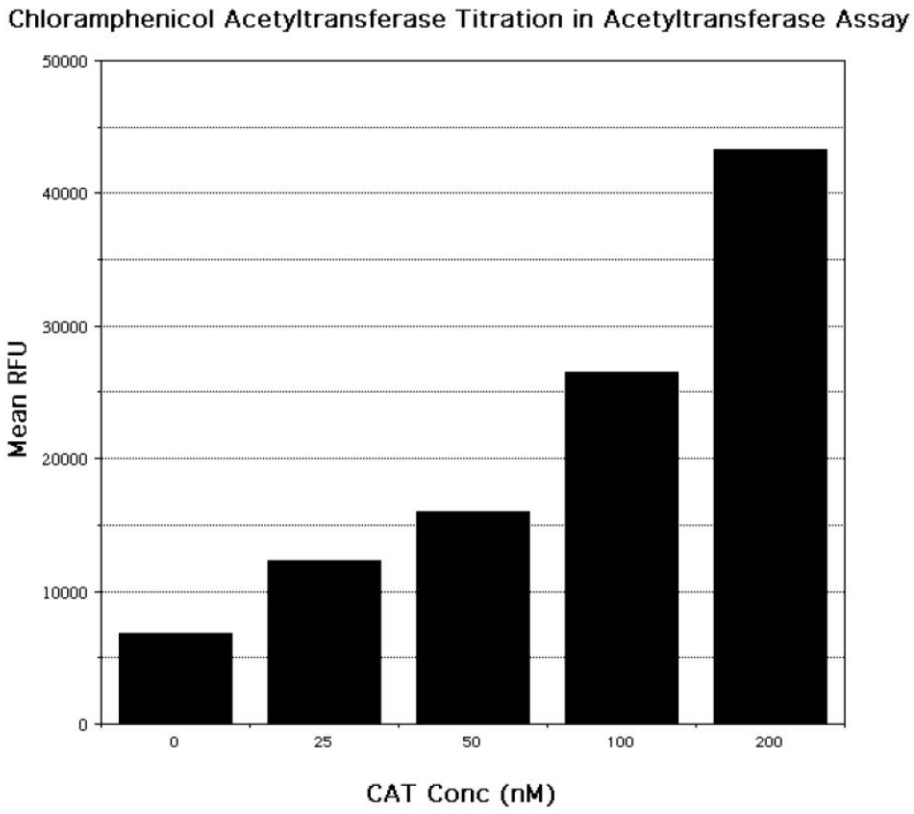


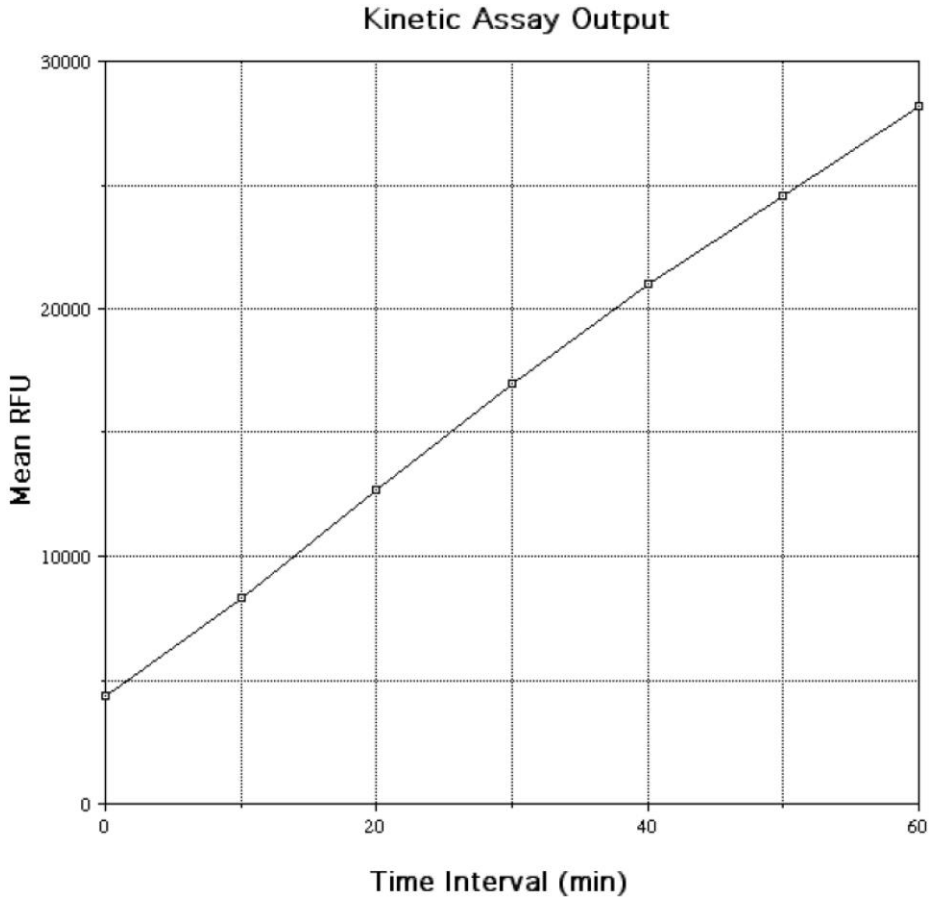
Figure 1:

Chloramphenicol acetyltransferase (CAT) was titered in the assay using 100µM of the Substrate chloramphenicol. Serial dilutions of the enzyme were prepared in Transferase Assay Buffer. Mean relative fluorescence was plotted against CAT concentration to generate the following graph. This is for illustration purposes only. The investigator must titrate their enzyme / substrate system in the assay.

Based on this titration data, the acetyltransferase concentration of 100nM produces a maximum signal within the detection range of the plate reader, with a signal to noise ratio sufficient for easy detection of altered enzyme activity.

Example Kinetic Assay Results

100nM CAT was tested with 100µM Chloramphenicol substrate in the kinetic assay format. Mean relative fluorescence was plotted against the stop time interval to generate this graph. This graph is for illustration purposes only. The kinetic assay must be optimized by the investigator, with their enzyme / substrate system.



Typical Inhibition Assay Procedure

Refer to the Assay Layout Sheet to determine the number of wells to be used. Cover unused wells tightly with a plate sealer. **DO NOT REUSE WELLS!**

1. Pipet 25µL of 1X Transferase Assay Buffer into the blank wells.
2. Pipet 10µL of 1X Transferase Assay Buffer into the zero wells.
3. Pipet 10µL of inhibitor dilution into the bottom of the appropriate wells.
4. Pipet 15µL of acetyltransferase at chosen working concentration into appropriate wells.
5. Cover plate with foil plate sealer. Incubate for 10 min at room temperature without shaking.
6. Pipet 25µL of 1X Reaction Mix into each well.
7. Cover plate with foil plate sealer. Incubate for 30 min shaking* at room temperature.
8. Pipet 50µL of ice cold isopropyl alcohol into each well.
9. Pipet 100µL of 1X Detection Solution into each well.
10. Cover plate with foil plate sealer. Incubate for 10 min at room temperature without shaking.
11. Read fluorescence at 380ex/520em.

* Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.

Calculation of Results

Several options are available for the calculation of the inhibition of acetyltransferase. We recommend that the data be handled by a software package utilizing a suitable curve fitting program to determine the percent inhibition. If data reduction software is not readily available, the data can be calculated as follows:

1. Calculate the mean net RFU for each sample by subtracting the mean blank RFU from the mean RFU for the samples:

   Mean Net RFU = Mean Sample RFU - Mean Blank RFU

2. Percent inhibition should be calculated using the following formula for each inhibitor dilution:

   Percent Inhibition =

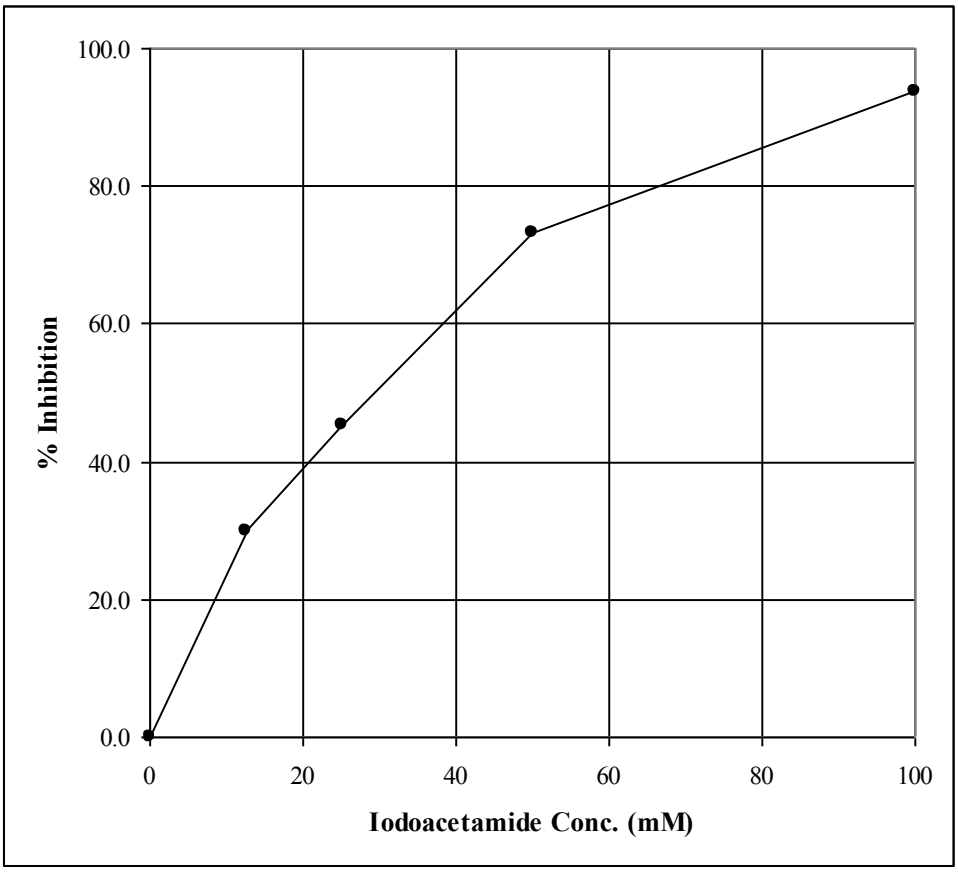
$$\frac{(\text{Mean Net Zero (non-inhibited enzyme) RFU} - \text{Mean Net Inhibited enzyme RFU})}{\text{Mean Net Zero (non-inhibited enzyme) RFU}} \times 100$$

Typical Inhibition Assay Results

Using Chloramphenicol Acetyltransferase (CAT) and Chloramphenicol as an enzyme substrate system, percent inhibition for dilutions of Iodoacetamide was tested. The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Dilution	Inhib. Conc. (mM)	Mean RFU	Mean Net RFU	% Inhibition
1	100mM	8,707	2,161	93.6%
2	50mM	15,575	9,029	73.1%
3	25mM	24,951	18,405	45.2%
4	12.5mM	30,110	23,564	29.8%
0	0mM	40,127	33,581	0%
blank	--------	6,889	0	-------

Typical Inhibition Curve



Typical Enzyme Interference Assay Procedure

*Prepare dilutions of the colored compound to be screened in 1X Transferase Assay Buffer.

1. Pipet 50µL of 1X Transferase Assay Buffer into the blank wells.
2. Pipet 25µL of 1X Transferase Assay Buffer into the zero wells.
3. Pipet 25µL of colored compound dilution into appropriate wells
4. Pipet 25µL of 1X Positive Control into the zero wells, and wells containing colored compound dilutions.
5. Pipet 50µL of ice cold isopropyl alcohol into each well.
6. Pipet 100µL 1X Detection Solution into each well.
7. Cover plate with foil plate sealer, incubate for 10 min at room temperature without shaking.
8. Read fluorescence at 380ex/520em.

To determine whether or not colored compounds will interfere with the assay, calculate and compare the signal to noise ratio of the colored compound dilutions to the signal to noise ratio of the zero wells.

Interfering Substances

The following solvents were tested for interference with the fluorescent signal generated in the assay. The table lists the percentage of signal in the presence of interferant relative to the zero for each solvent.

% Interferant	DMSO	DMF	Acetonitrile
12.5	108	68	49
6.25	107	82	71
3.12	107	88	86
1.56	112	98	96
0.78	114	104	95
0.39	115	109	100
0	100	100	100

* Percent interferant is relative to a 50 µL total reaction volume.

Diluents containing bovine serum albumin (BSA), or other Thiol containing reagents, should be treated with N-Ethylmaleimide (NEM) prior to use in the assay. We recommend reacting 10% BSA with 1 mM NEM for 1 hour at room temperature, however this procedure should be optimized for each reagent.

References

1. Walsh, C.T.. Posttranslational Modification of Proteins, Expanding Nature's Inventory. Roberts and Company; Englewood.

2. Schilz, R.L., et. al. Overlapping but Distinct Patterns of Histone Aceytlation by the Human Coactivators p300 and PCAF within Nucleosomal Substrates. J. Biol. Chem. (1999); 274(3): 1189-1999.

3. Worbly, C.A. & D.E. Dixon. Bacteria Seize Control b y Acetylating Host Proteins. Science (2006) 312:1150-1151.

4. Kim, M.Y.,et. al. A Role for Coactivators and Histone Acetylation in Estrogen Receptor α-mediated Transcription Initiation. EMBO J (2001) 20(21):6084-6094.

5. DeAngelis, J., et. al. Kinetic Analysis if the Catalytic Mechanism of Serotonin N-Acetyltransferase (EC 2.3.1.87). J. Biol. Chem. (1998) 273(5):3045-3050.

6. Fitton, J.E. & W.V. Shaw. Comparison of Chloramphenicol Aceyltransferase Variants in staphylococci; Purification, Inhibitor Studies and N-terminal Sequences. Biochem. J. (1997) 177:575-582.

7. Tai, H.H. et. al. Dynamic Histone Acetylation of Late Embryonic Genes During Seen Germination. Plant Mol. Biol. (2005) 59(6):909-925.

8. Khalil, E.M., et. al. Mechanism-based Inhibition of the Melatonin Rhythm Enzyme: Pharmacologic Exploitation of Active Site Functional Plasticity. PNAS (1999); (22):12418-12423.

9. Brockton, N. et. al. N-Acetyltransferase Polhymorphisms and Colorectal Cancer: A HuGe Review. J. Epidem. (2000) 151(9):846-861.

10. Shi, H., et. al. Tripple Analaysis of the Cancer Epigenome: An Integrated Microarray System for Assessing Gene Expression, DNA Methylation, and Histone Aceylation. Cancer Res. (2003) 63:2164-2171.

Notes

Notes

MSDS (Material Safety Data Sheet) available online

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