ab284533 – Arylamine N-acetyltransferase Activity Assay Kit (Fluorometric)

For the measurement of NAT activity in tissue samples or purified protein.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284533

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, store the kit components as per the respective temperatures mentioned below. Use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
NAT Assay Buffer	25 mL	-20°C
NAT Substrate I	200 µL	-20°C
NAT Substrate II	2 vials	-20°C
DTT	100 µL	-20°C
Acetylated Standard	50 µL	-20°C
NAT Positive Control	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well, white plate with flat bottom
- Multi-well spectrophotometer
- DMSO
- Deionized water
- Dounce Tissue Homogenizer

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

NAT Assay Buffer: Warm to room temperature (RT) before use.

NAT Substrate I and Acetylated Standard (10 mM): Thaw at RT and keep at RT when in use.

NAT Substrate II: Reconstitute 1 vial at a time with 1.1 ml deionized water. Divide into aliquots and store at -80°C. Stable at -80°C for at least two months. Avoid repeated freeze/thaw cycles. Keep on ice while in use.

DTT (1 M): Avoid repeated freeze/thaw cycles. Keep on ice when in use.

NAT Positive Control: Reconstitute in 22 µl NAT assay buffer. Divide into aliquots and store at -20°C. Keep on ice when in use.

NAT Activity Assay Protocol

Sample Preparation:

1. Homogenize tissue (100 mg) with 400 µl NAT Assay buffer using Dounce Tissue Homogenizer. Keep on ice for 10 min.
2. Prepare tissue S9 fraction by centrifuging at 9,000 x g and 4°C for 20 min. Collect the supernatant (S9) and estimate the protein concentration using any preferred method. We recommend using BCA Protein Assay Kit. Protein concentration should range between 5 - 20 µg/µl. Dilute the lysate if needed using NAT Assay Buffer.
3. Prepare two wells for each Sample to be tested labeled as Sample (S) and Sample Background Control (SBC). Add 2-8 µl Sample(s) (up to 160 µg protein) into each of these wells.
4. For Positive Control, add 2-4 µl of the reconstituted NAT Positive Control into the desired well (s).
5. Adjust the volume of S, SBC, and Positive Control wells to 50 µl/well with NAT Assay Buffer.
6. For Substrate Control (SC) wells, add 50 µl of NAT Assay Buffer.

Δ Note: *We recommend using the Samples for activity analysis immediately. Otherwise, store the Sample(s) at -80°C for 3-4 days.*
Δ Note: *For Unknown Samples, we suggest testing several concentrations to ensure that the readings are within the Standard Curve range.*

Standard Curve Generation:

1. Dilute the provided Acetylated Standard at 1:10 dilution in DMSO to obtain 1 mM Acetylated Standard.
2. Dilute the 1 mM Acetylated Standard further (1:20 dilution) in DMSO to obtain a 50 µM Acetylated Standard solution.
3. Add 0, 2, 4, 6, 8, 10 µl of the 50 µM Acetylated Standard into a 96-well, white plate to generate 0, 100, 200, 300, 400, 500 pmol/well of Acetylated Standard.
4. Adjust the volume of each Standard well to 100 µl with NAT Assay Buffer.

Reaction Mix Preparation:

1. Dilute the stock DTT at 1:10 dilution in NAT assay buffer to obtain DTT working solution. Do not dilute the whole vial at one time).
2. Mix enough reagents for the number of assays to be performed.
3. For each well, prepare a total of 50 µl Mix as mentioned in the table below:

	Reaction Mix	SBC Mix
NAT Assay Buffer	26 µL	46 µL
NAT Substrate I	2 µL	2 µL
NAT Substrate II	20 µL	---
DTT (working solution)	2 µL	2 µL

4. Mix well and add 50 µL of Reaction Mix to Substrate Control, Sample(s), and Positive Control wells and SBC Mix to "Sample Background Control" wells respectively.

Δ Note: *Have the plate reader ready at Ex/Em = 360/440 nm in kinetic mode at 37°C set to record fluorescence every 30 seconds.*
Δ Note: *Prepare reaction mix immediately before adding to wells.*

Measurement

1. Immediately start recording fluorescence at 30 sec intervals for 15-30 min at 37°C.
2. Samples with low activity may be run for 30-60 min.
3. Standard Curve may be read in end point mode.

Calculation:

1. Subtract the 0 Standard reading from all Standard readings and SBC reading from all Sample readings respectively. If the Substrate Control reading is higher than the SBC reading, subtract the Substrate Control readings from the Sample readings instead.

2. Plot the Acetylated Standard Curve.
3. Choose any two time points within the linear portion of the curve (t1 and t2) for each Sample type. Use the Acetylated Standard Curve to estimate the amount of acetylated product formed between t2 and t1 during the enzymatic reaction for each of the Samples.
4. Calculate ΔM, which is the change in amount of acetylated product formed between t2 and t1 (Δt2 - t1).
5. NAT activity may be calculated using the following equation:

Sample NAT Specific Activity = ΔM / (Δt x P) (pmol / (min x µg)) = µUnits / µg or mUnits / mg

Where:
ΔM = Amount of acetylated product formed during Δt (pmol)
Δt = t2 - t1 (min)
P = Sample protein amount added per well (µg)

Unit Definition: One unit of NAT is the amount of enzyme that produces1 µmol of acetylated product per minute at pH 7.5 at 37°C.

Technical Support

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