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Arylamine N-acetyltransferase Activity Assay Kit (Fluorometric)

rev 12/19

(Catalog # K2031-100; 100 assays; Store at -20ºC)

I. Introduction:

Arylamine N-acetyltransferases (NATs, EC 2.3.1.5) are a group of cytosolic, conjugating enzymes involved in phase II xenobiotic metabolism. They transfer an acetyl group from acetyl Coenzyme A to a xenobiotic acceptor substrate. There are two main isoforms, NAT1 and NAT2, which share about 81% amino acid sequence homology. NAT1 is widely expressed in all tissues whereas NAT2 is present primarily, in the liver and intestine. In addition to its role in xenobiotic metabolism, NAT1 plays an important role in folate metabolism. NAT2 is a polymorphic enzyme wherein the slow acetylator phenotype of NAT2 has been linked to urinary bladder cancer whereas the rapid acetylator phenotype has been linked to colorectal cancer. The slow acetylator phenotype has also been widely linked with toxicity due to isoniazid: a widely used tuberculosis drug. **BioVision's Arylamine N-acetyltransferase Activity Assay Kit** is a simple, one step plate based assay that measures a fluorescent product. It can measure both NAT1 and NAT2 activity and can detect as low as 1 µU in samples.

$$\text{Arylamine substrate + Acetyl CoA} \xrightarrow{\text{NAT}} \text{CoA + Acetylated arylamine (Fluorescence (Ex/Em = 360/440 nm)}$$

II. Applications:

Measurement of NAT activity in tissue samples or purified protein

III. Sample Type:

- S9 fractions
- Recombinant enzyme
- Purified protein

IV. Kit Contents:

Components	K2031-100	Cap Code	Part Number
NAT Assay Buffer	25 ml	NM	K2031-100-1
NAT Substrate I	200 µl	White	K2031-100-2
NAT Substrate II	2 vials	Red	K2031-100-3
DTT	100 µl	Blue	K2031-100-4
Acetylated Standard	50 µl	Yellow	K2031-100-5
NAT Positive Control	1 vial	Green	K2031-100-6

V. User Supplied Reagents and Equipment:

- 96-well, white plate with flat bottom
- Multi-well spectrophotometer
- DMSO
- Deionized water
- Dounce Tissue Homogenizer (BioVision Cat # 1998)

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge the small vials before opening. Read the entire protocol before performing the assay. Components are stable for at least three months.

- **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 -20ºC. Keep on ice when in use.

VII. NAT Activity Assay Protocol:

1. Sample preparation: Homogenize tissue (100 mg) with 400 µl NAT Assay buffer using Dounce Tissue Homogenizer (BioVision Cat # 1998). Keep on ice for 10 min. Prepare tissue S9 fraction by centrifuging at 9,000 x *g* and 4ºC for 20 min. Collect the supernatant (S9) and estimate the protein concentration using any preferred method. We recommend using BCA Protein Assay Kit (BioVision Cat # K813-2500). Protein concentration should range between 5-20 µg/µl. Dilute the lysate if needed using NAT Assay Buffer. 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. For Positive Control, add 2-4 µl of the reconstituted NAT Positive Control into the desired well(s). Adjust the volume of S, SBC and Positive Control wells to 50 µl/well with NAT Assay Buffer. For Substrate Control **(SC)** wells, add 50 µl of NAT Assay Buffer.

Notes:

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

2. Standard Curve Generation: Dilute the provided Acetylated Standard at 1:10 dilution in DMSO to obtain 1 mM Acetylated Standard. Dilute the 1 mM Acetylated Standard further (1:20 dilution) in DMSO to obtain a 50 µM Acetylated Standard solution. 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. Adjust the volume of each Standard well to 100 µl with NAT Assay Buffer.

3. **Reaction Mix Preparation:** 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)***. Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 50 µl Mix as mentioned 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

Mix well. Add 50 µl Reaction Mix to Substrate Control, Sample(s), and Positive Control wells and SBC Mix to "Sample Background Control" wells respectively.

Notes:

a. Have the plate reader ready at Ex/Em = 360/440 nm in kinetic mode at 37ºC set to record fluorescence every 30 sec.

b. Prepare reaction mix immediately before adding to wells.

4. **Measurement:** Immediately start recording fluorescence at 30 sec intervals for 15-30 min at 37ºC. Samples with low activity may be run for 30-60 min. Standard Curve may be read in end point mode.
5. **Calculation:** 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. Plot the Acetylated Standard Curve. Choose any two time points within the linear portion of the curve (t_1 & t_2) for each Sample type. Use the Acetylated Standard Curve to estimate the amount of acetylated product formed between t_2 and t_1 during the enzymatic reaction for each of the Samples. Calculate ΔM, which is the change in amount of acetylated product formed between t_2 and t_1 (Δt_2 - t_1).
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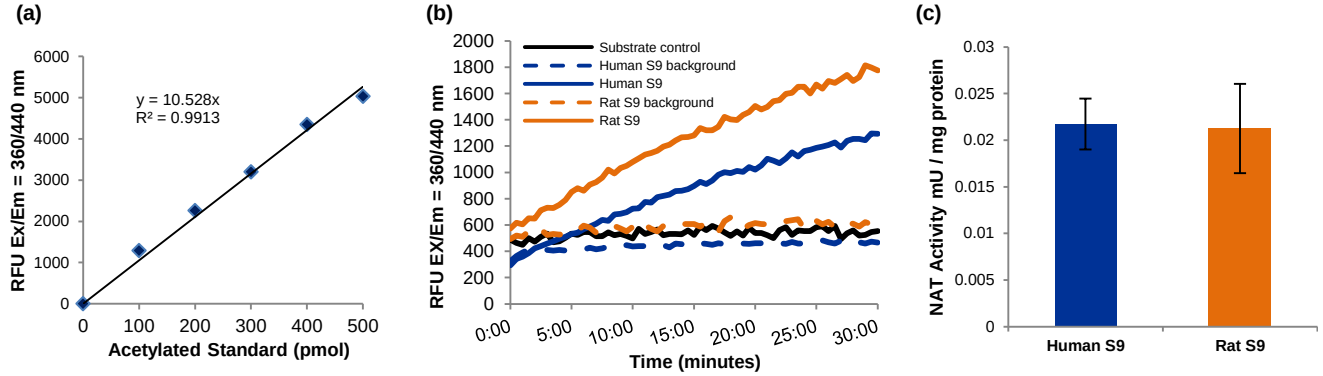
NAT activity may be calculated using the following equation**:**

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 = $t_2 - t_1$ (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 37ºC.



Figures: (**a**) Acetylated Standard Curve (**b**) Enzyme kinetics using Human Liver S9 fraction (160 µg protein/well) and Rat Liver S9 fraction (120 µg protein/well) **(c)** Arylamine N-acetyltransferase specific activity in human and rat liver S9 fractions. Experiments were conducted according to kit protocol.

VIII. Related Products:

Cytochrome P450 2C19 (CYP2C19) Activity Assay Kit (Fluorometric) (K848)
Cytochrome P450 2C9 (CYP2C9) Activity Assay Kit (Fluorometric) (K895)
Cytochrome P450 1A2 (CYP1A2) Activity Assay Kit (Fluorometric) (K893)

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