Technical Bulletin

Arylamine N-acetyltransferase Activity Assay Kit (Fluorometric)

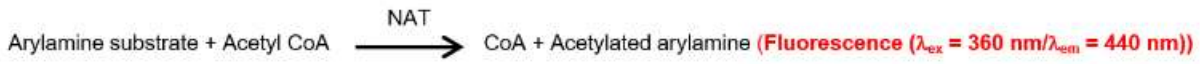
Catalog Number MAK430

Product Description

Arylamine N-acetyltransferases (NATs) 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 in which the slow acetylator phenotype of NAT2 has been linked to urinary bladder cancer, while the rapid acetylator phenotype has been linked to colorectal cancer. The slow acetylator phenotype of NAT2 has also been widely linked with toxicity due to isoniazid, a widely used tuberculosis drug.

The Arylamine N-acetyltransferase Activity Assay Kit is a simple, one step plate-based assay that measures a fluorescent product. The method can measure both NAT1 and NAT2 activity and can detect as low as 1 μU of activity in samples.

The kit is suitable for the measurement of NAT activity in tissue samples or purified protein.



Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- NAT Assay Buffer — 25 mL
  Catalog Number MAK430A
- NAT Substrate I — 200 μL
  Catalog Number MAK430B
- NAT Substrate II — 2 x 1 vial
  Catalog Number MAK430C
- DTT (1 M) — 100 μL
  Catalog Number MAK430D
- Acetylated Standard — 50 μL
  Catalog Number MAK430E
- NAT Positive Control — 1 vial
  Catalog Number MAK430F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Dimethyl Sulfoxide (DMSO), anhydrous (Catalog Number 276855 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥9,000 × *g*
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

NAT Assay Buffer: Warm to room temperature prior to use.

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

NAT Substrate II: Reconstitute 1 vial at a time with 1.1 mL of purified 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 vial in 22 µL of NAT Assay Buffer. Divide into aliquots and store at -20 °C. Keep on ice when in use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Homogenize tissue (100 mg) with 400 µL of NAT Assay Buffer using Dounce Tissue Homogenizer.
2. Keep homogenate on ice for 10 minutes.
3. Prepare tissue S9 fraction by centrifuging at 9,000 × *g*, 4 °C for 20 minutes.
4. Collect the supernatant (S9) and estimate the protein concentration using any preferred method. BCA protein determination is recommended. Protein concentration should range between 5-20 µg/µL. Dilute the lysate if needed using NAT Assay Buffer. Use the samples for activity analysis immediately. Otherwise, store at -80 °C for 3-4 days.
5. 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 unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.
6. Adjust the total volume of all wells to 50 µL/well with NAT Assay Buffer.

Substrate Control (SC)

For Substrate Control wells, add 50 µL of NAT Assay Buffer.

Positive Control

Add 2-4 µL of the reconstituted NAT Positive Control into the desired well(s). Adjust the total volume to 50 µL/well with NAT Assay Buffer.

Standard Curve Preparation

1. Dilute the provided Acetylated Standard (10 mM) 1:10 in DMSO (not included) to obtain a 1 mM Acetylated Standard.
2. Dilute the 1 mM Acetylated Standard from Step 1 1:20 in DMSO to obtain a 50 µM Acetylated Standard solution. Prepare Acetylated Standards according to Table 1. Mix well.

Table 1.
Preparation of Acetylated Standards

Well	50 µM Acetylated Standard	NAT Assay Buffer	Acetylated Standard (pmol/well)
1	0 µL	100 µL	0
2	2 µL	92 µL	100
3	4 µL	96 µL	200
4	6 µL	94 µL	300
5	8 µL	92 µL	400
6	10 µL	90 µL	500

Reaction Mix

1. Set the plate reader at λ_{Ex} = 360 nm/ λ_{Em} = 440 nm in kinetic mode at 37 °C and to record fluorescence every 30 seconds **prior** to preparing reaction mixes.
2. Dilute an aliquot of the stock DTT 1:10 in NAT Assay Buffer to obtain DTT working solution. **Only dilute the amount needed, do not dilute the entire vial at one time**.
3. Mix enough reagents for the number of assays to be performed. Prepare reaction mixes immediately prior to adding to wells.
   a. For each well containing Sample (S), Substrate Control (SC), or Positive Control (PC), prepare 50 µL of Sample Reaction Mix according to Table 2, mix well.
   b. For each Sample Background Control well (SBC), prepare SBC Reaction Mix according to Table 2, mix well.

Table 2.
Preparation of Reaction Mixes

Reagent	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. Add 50 µL of Reaction Mix to Substrate Control (SC), Sample(s) (S), and Positive Control (PC) wells and 50 µL of SBC Mix to Sample Background Control (SBC) wells respectively.

Measurement

Immediately start recording fluorescence at 30 second intervals for 15-30 minutes at 37 °C. Samples with low activity may be run for 30-60 minutes. The Standard Curve may be read in end point mode.

Results

1. Subtract the 0 Standard reading from all Standard readings. Subtract the SBC reading from all Sample readings.
   a. If the Substrate Control (SC) reading is higher than the SBC reading, subtract the Substrate Control readings from the Sample readings instead of the SBC reading.
2. Plot the Acetylated Standard Curve.
3. Choose two time points within the linear portion of the curve (T_1 and T_2) for each Sample type.
4. 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.
5. Calculate ΔM, which is the change in amount of acetylated product formed between T_2 and T_1 ($\Delta T = T_2 - T_1$).

6. NAT activity may be calculated using the following equation:

Sample NAT Specific Activity (pmol/(min × µg)) or µUnits/µg or mUnits/mg) =

$$[\Delta M / (\Delta T \times P)]$$

where

ΔM = Amount of acetylated product formed during ΔT (pmol)

ΔT = $T_2 - T_1$ (Reaction time in minutes)

P = Sample protein amount added per well (µg)

Unit Definition: One unit of NAT is the amount of enzyme that produces 1 µmol of acetylated product per minute at pH 7.5 at 37 °C.

Figure 1.
Typical Acetylated Standard Curve

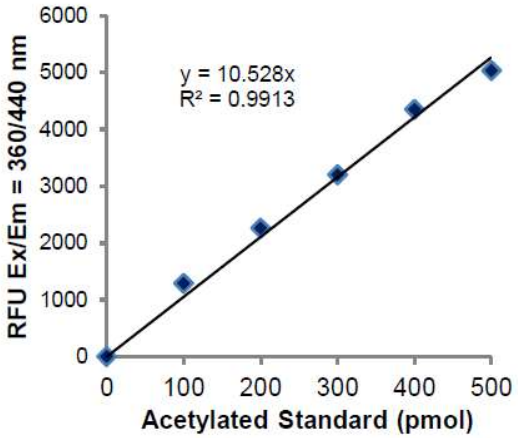


Figure 2.
Enzyme kinetics using Human Liver S9 fraction (160 µg protein/well) and Rat Liver S9 fraction (120 µg protein/well)

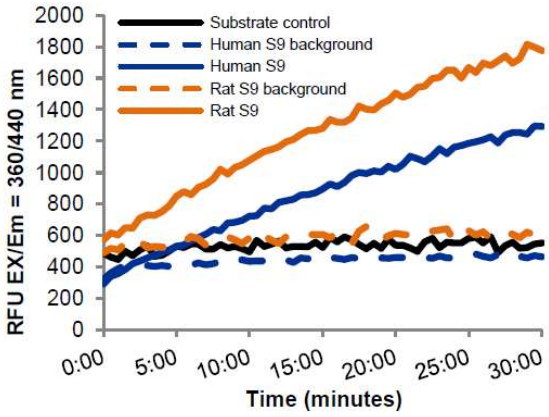
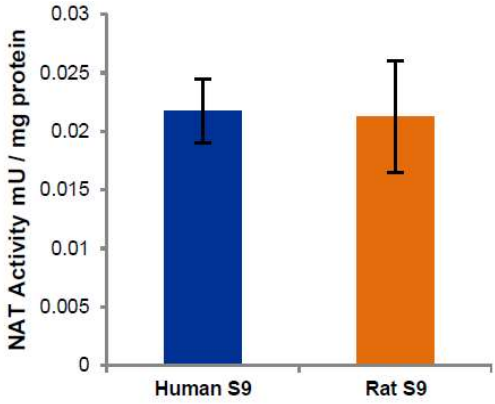


Figure 3.
Arylamine N-acetyltransferase specific activity in human and rat liver S9 fractions. Experiments were conducted according to kit protocol.



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