ab284541 – N-Acetylcysteine Assay Kit (Fluorometric)

For determining the concentration of N-Acetylcysteine in biological samples and pharmaceuticals

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284541

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
N-Acetylcysteine Assay Buffer	50 mL	-20°C
N N-Acetylcysteine Enzyme Mix I	2 vials	-20°C
N-Acetylcysteine Enzyme Mix II	3 vials	-20°C
Reducing Agent	3 vials	-20°C
N-Acetylcysteine Blocker	100 µL	-20°C
N-Acetylcysteine Probe	0.5 mL	-20°C
N-Acetylcysteine Standard	1 vial	-20°C
87% TCA Solution	3 mL	-20°C
Neutralization Solution	4 mL	-20°C

Materials Required, Not Supplied

- dH2O
- 96-well black plate with flat bottom (low/medium binding)
- Multi-well spectrophotometer (Fluorescent plate reader)

Reagent Preparation

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

N-Acetylcysteine Assay Buffer: Warm to room temperature (RT) before use.

N-Acetylcysteine Enzyme Mix I: Reconstitute each vial with 110 µL N-Acetylcysteine Assay Buffer. Divide into aliquots and store at -20°C. Keep on ice during use.

N-Acetylcysteine Enzyme Mix II: Reconstitute each vial with 1 mL N-Acetylcysteine Assay Buffer. Keep on ice during use. Store at 4°C. Use the reconstituted N-Acetylcysteine Enzyme Mix II within a week.

Reducing Agent: Reconstitute each vial with 220 µL N-Acetylcysteine Assay Buffer. Keep on ice during use. The remaining solution can be kept at 4°C for 1 week.

N-Acetylcysteine Blocker: Bring to RT. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles.

N N-Acetylcysteine Probe (in DMSO): Ready to use. Divide into aliquots and store at -20°C, protected from light. Warm solution to RT before performing the assay.

N-Acetylcysteine Standard: Reconstitute each vial in 1.1 mL dH2O. Aliquot and store at -20°C. Warm to RT before use.

87% TCA Solution & Neutralization Solution: Store at RT. Place the components on ice to chill before use

Assay Protocol

Sample preparation:

1. Centrifuge the sample(s) at 12,000 x g, 4°C for 10 min to remove any insoluble materials. Collect the supernatant into a new tube.
2. Add 10 µL cold TCA Solution to 200 µL supernatant to deproteinize the molecules that might interfere with the assay.
3. Keep on ice for 15 min, centrifuge at 12,000 x g, 4°C for 5 min. Carefully transfer the supernatant (~150 µL) into another tube and add 5 µL cold Neutralizing Solution.
4. Mix well and place on ice for 5 min.
5. Deproteinized and neutralized sample (s) are used for the assay. Add 5 µL Sample(s) into duplicate wells of a black, 96-well plate labeled as Sample Background Control [SBC], and Sample [S].
6. Adjust the volume to 100 µL/well for SBC and 98 µL/well for Sample(s) using N-Acetylcysteine Assay Buffer.

Δ Notes:

a) *We recommend using the samples immediately. Store the Sample(s) at -80°C for future experiments.*

b) *For Unknown Samples, we suggest testing several dilutions of your samples to ensure that the readings are within the Standard Curve range. Samples with higher levels of N-Acetylcysteine may be diluted with N-Acetylcysteine Assay Buffer.*

Standard Curve Generation

1. Prepare 10-fold dilution of the N-Acetylcysteine Standard by adding 10 µL of the reconstituted N-Acetylcysteine Standard to 90 µL of dH2O.
2. Add 0, 2, 4, 6, 8 and 10 µL of the diluted N-Acetylcysteine Standard into a series of wells of a 96-well black plate to generate 0, 2, 4, 6, 8 and 10 nmol/well N-Acetylcysteine Standard.
3. Adjust the volume of each well to 98 µL with N-Acetylcysteine Assay Buffer. Mix well.

N-Acetylcysteine Enzyme Mix I

1. Add 2 µL of reconstituted N-Acetylcysteine Enzyme Mix I into Standard and Sample(s) wells.

Reaction Mix Preparation

1. Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 100 µL Reaction Mix containing:

	Reaction Mix
N-Acetylcysteine Assay Buffer	98 µL
Reducing Agent	1 µL
N-Acetylcysteine Blocker	1 µL

2. Mix well.
3. Add 100 µL Reaction Mix to all the wells including Standard, Sample(s) and SBC wells. Gently mix by tapping the plate.
4. Incubate the reaction at 37°C for 30 min.

N-Acetylcysteine Enzyme Mix II Addition:

1. Add 30 µL of reconstituted N-Acetylcysteine Enzyme Mix II into all wells including Standard, Sample(s) and SBC wells.

2. Gently mix and incubate at 37°C for 5 min.

Δ Notes:

a) *Follow the protocol exactly as described. Any deviations can result in sub-optimal results.*

b) *Incubation time for both the Standard and the Sample wells must be consistent.*

Measurement

After incubation, add 5 µL N-Acetylcysteine probe. Gently mix and measure the fluorescence of all wells at 37°C (Ex/Em = 368/460 nm) in kinetic mode at 1 min intervals for at least 30 min**. The final reaction volume in all wells is 235 µL.**

Calculation

1. For N-Acetylcysteine Standard Curve, subtract the 0 Standard RFU reading (0 nmol/well) from all Standard RFU readings and plot the N-Acetylcysteine Standard Curve.
2. For both Sample(s) and SBC, choose any two time points within the linear range of the curve (t_1 & t_2) and calculate the respective slopes.
3. Subtract the slope of SBC from the Sample(s) slope.
4. Apply the corrected Sample(s) slope values to the N-Acetylcysteine Standard Curve to get nmol of N-Acetylcysteine in the Sample(s).

N-Acetylcysteine Concentration in Sample(s) = $\frac{B}{V}$ **x D = nmol/µl = mM**

Where:
B is the amount of N-Acetylcysteine calculated from the N-Acetylcysteine Standard Curve (nmol)
V is the volume of Sample added to the well (µL)
D is the Sample dilution factor (D = 1 for Undiluted Samples)

Technical Support

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