Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

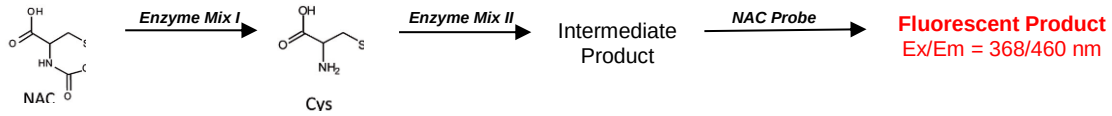
N-Acetylcysteine Assay Kit (Fluorometric)

05/20

(Catalog # K2044-100; 100 assays; Store at -20°C)

I. Introduction:

N-Acetylcysteine (NAC) is a precursor of L-cysteine and glutathione biosynthesis. It is a powerful antioxidant and a scavenger of free radicals. It has been commonly used for treating cough, flu, dry eye, lung conditions, paracetamol intoxication etc. Additionally, recent investigations have explored the beneficial effects of NAC in type 2 diabetes and psychiatric disorders. NAC is available as over the counter in tablet, solution as well as intravenous and inhaled preparations. Common side effects of NAC treatment are generally mild and typically resolve on their own once the treatment is stopped. **BioVision's N-Acetylcysteine Assay Kit** is a simple and sensitive method for the quantitation of NAC in biological samples. The assay is based on the deacetylation reaction of NAC to generate cysteine. Cysteine is then catalyzed in subsequent reactions thereby producing an intermediate product, which reacts with a fluorogenic probe to form a stable fluorophore measured at Ex/Em = 368/460 nm. The assay is specific with no interference from other thiol-based amino acids.



II. Application:

- Determining the concentration of N-Acetylcysteine in biological samples and pharmaceuticals.

III. Sample Type:

- Biological samples (serum, plasma etc.)

IV. Kit Contents:

Components	K2044-100	Cap Code	Part Number
NAC Assay Buffer	50 ml	WM	K2044-100-1
NAC Enzyme Mix I	2 vials	Red	K2044-100-2
NAC Enzyme Mix II	3 vials	Blue	K2044-100-3
Reducing Agent	3 vials	Yellow	K2044-100-4
NAC Blocker	100 µl	White	K2044-100-5
NAC Probe	0.5 ml	Purple	K2044-100-6
NAC Standard	1 vial	Green	K2044-100-7
87% TCA Solution	3 ml	NM	K2044-100-8
Neutralization Solution	4 ml	NM	K2044-100-9

V. User Supplied Reagents and Equipment:

- dH_2O
- 96-well black plate with flat bottom (low/medium binding)
- Multi-well spectrophotometer (Fluorescent plate reader)

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

- **NAC Assay Buffer:** Warm to room temperature (RT) before use.
- **NAC Enzyme Mix I:** Reconstitute each vial with 110 µl NAC Assay Buffer. Divide into aliquots and store at -20°C. Keep on ice during use.
- **NAC Enzyme Mix II:** Reconstitute each vial with 1 ml NAC Assay Buffer. Keep on ice during use. Store at 4°C. Use the reconstituted NAC Enzyme Mix II within a week.
- **Reducing Agent:** Reconstitute each vial with 220 µl NAC Assay Buffer. Keep on ice during use. The remaining solution can be kept at 4°C for 1 week.
- **NAC Blocker:** Bring to RT. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles.
- **NAC 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.
- **NAC Standard:** Reconstitute each vial in 1.1 ml dH_2O. 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.

VII. N-Acetylcysteine Assay Protocol:

1. Sample Preparation: 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. Add 10 µl cold TCA Solution to 200 µl supernatant to deproteinize the molecules that might interfere with the assay. 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. Mix well and place on ice for 5 min. 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].** Adjust the volume to 100 µl/well for SBC and 98 µl/well for Sample(s) using NAC Assay Buffer.

Notes:

a. We recommend using the samples immediately. Store the Sample(s) at -80ºC 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 NAC may be diluted with NAC Assay Buffer.

2. **Standard Curve Preparation:** Prepare 10 fold dilution of the NAC Standard by adding 10 µl of the reconstituted NAC Standard to 90 µl of dH_2O. Add 0, 2, 4, 6, 8 and 10 µl of the diluted NAC Standard into a series of wells of a 96-well black plate to generate 0, 2, 4, 6, 8 and 10 nmol/well NAC Standard. Adjust the volume of each well to 98 µl with NAC Assay Buffer. Mix well.
3. **NAC Enzyme Mix I:** Add 2 µl of reconstituted NAC Enzyme Mix I into Standard and Sample(s) wells.
4. **Reaction Mix Preparation:** 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
NAC Assay Buffer	98 µl
Reducing Agent	1 µl
NAC Blocker	1 µl

Mix well and add 100 µl Reaction Mix to all the wells including Standard, Sample(s) and SBC wells. Gently mix by tapping the plate. Incubate the reaction at 37ºC for 30 min.

5. **NAC Enzyme Mix II Addition:** Add 30 µl of reconstituted NAC Enzyme Mix II into all wells including Standard, Sample(s) and SBC wells. Gently mix and incubate at 37ºC for 5 min.

Notes:

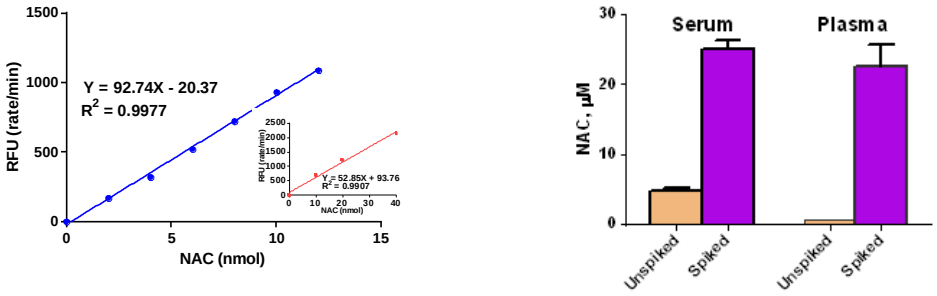
a. Follow the protocol exactly as described. Any deviations can result in sub-optimal results.
b. Incubation time for both the Standard and the Sample wells must be consistent.

6. **Measurement:** After incubation, add 5 µl NAC probe. Gently mix and measure the fluorescence of all wells at 37ºC (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***
7. **Calculation:** For NAC Standard Curve, subtract the 0 Standard RFU reading (0 nmol/well) from all Standard RFU readings and plot the NAC Standard Curve. 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. Subtract the slope of SBC from the Sample(s) slope. Apply the corrected Sample(s) slope values to the NAC Standard Curve to get nmol of NAC in the Sample(s).

$$\textbf{NAC Concentration in Sample(s)} = \frac{B}{V} \times D = \text{nmol/µl} = \text{mM}$$

Where **B** is the amount of NAC calculated from the NAC 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)

(a) **(b)**



Figures: (a) NAC Standard Curve. **(b)** Estimation of NAC in single donor human serum and plasma (5 µl), spiked with 5 nmol of NAC Standard (equivalent to 20 µM free NAC). Total NAC concentrations for serum and plasma were 4.8 ± 0.3 µM and 0.7 ± 0.0 µM with average spiked recoveries of 92.0% and 93.5%, respectively. The assay was performed according to the kit assay protocol.

VIII. Related Products:

N-Acetyl-L-Cysteine (Cat. # B1813-1G, 5G)
Cysteine Assay Kit (Fluorometric) (Cat. # K558-100)
Homocysteine α,γ-Lyase (rHCYase), Active, Recombinant (Cat. # P1117-200)
Homocysteine Assay Kit (Fluorometric) (Cat. # K531-100)
Methionine Assay Kit (Fluorometric) (Cat. # K442-100)
Deproteinizing Sample Preparation Kit (Cat. # K808-200)
Deproteinizing Sample Preparation Kit II (TCA method) (Cat. # K823-200)

FOR RESEARCH USE ONLY! Not to be used on humans.