Amplite™ Fluorimetric cADP-Ribose Assay Kit

Catalog number: 20305
Unit size: 100 tests

Component	Storage	Amount
Component A: Quest Fluor™ NAD Probe	Freeze (<-15 °C), Minimize light exposure	1 bottle (5 mL)
Component B: ADPRC Enzyme mix	Freeze (<-15 °C), Minimize light exposure	1 vial
Component C: Assay Solution I	Freeze (<-15 °C)	1 bottle (12 mL)
Component D: Assay Solution II	Freeze (<-15 °C)	1 bottle (5 mL)
Component E: Enhancer Solution	Freeze (<-15 °C), Minimize light exposure	1 bottle (3.5 mL)
Component F: cADPR Standard	Freeze (<-15 °C), Minimize light exposure	1 vial

OVERVIEW

cADP-ribose (cADPR) is a novel Ca2+ messenger derived from NAD+. ADP-riboxyl cyclase (ADPRC) catalyzes the synthesis of cADPR from NAD+, but the reaction can be reversed in the presence of high concentration of nicotinamide, producing NAD+ from cADPR stoichiometrically. The resultant NAD+ can be detected using our newly developed NAD sensor Quest Fluor™ NAD reagent. This makes monitoring cADPR in tissues and cell cultures possible in the low nM range. The NAD+ detection using Quest Fluor™ NAD reagent is specific to NAD+ and has no reaction to NADH. The fluorescent signal can be readily detected. This assay can be performed in a convenient 96-well or 384-well microtiter plate.

AT A GLANCE

Protocol summary

1. Prepare and add cADPR standards and/or test samples (50 µL)
2. Prepare and add ADRPC working solution (50 µL)
3. Incubate at room temperature for 1 hour
4. Add 40 µL Quest Fluor™ NAD Probe
5. Add 40 µL Assay Solution
6. Incubate at room temperature for 20 minutes
7. Add 30 µL Enhancer Solution
8. Incubate at room temperature for 20 minutes
9. Monitor fluoroscence intensity at Ex/Em = 420/480 nm

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	420 nm
Emission:	480 nm
Cutoff:	435 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

cADPR standard stock solution (5 mM):
Add 10 µL of ddH_2O into the vial of cADPR standard (Component F) and mix them well.

Note The unused cADPR stock solution should be stored at -20°C in single use aliquots.

PREPARATION OF STANDARD SOLUTION

cADPR standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/20305

Note Prepare cADPR serial dilutions in Assay Solution I (Component C).

PREPARATION OF WORKING SOLUTION

ADPRC working solution:
Add 50 µL ddH_2O into the vial of ADPRC Enzyme Mix (Component B) and mix well. Transfer whole content into 5 mL of Assay Solution I (Component C) and mix them well.

Note ADPRC working solution is not stable, use it promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of cADPR standards and test samples in a solid black 96-well microplate. ST = cADPR standard (ST1-ST7); BL = blank control; TS = test sample.

BL	BL	TS	TS
ST1	ST1	...	...
ST2	ST2	...	...
ST3	ST3		
ST4	ST4		
ST5	ST5		
ST6	ST6		
ST7	ST7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
ST1-ST7	50 µL	Serial Dilution
BL	50 µL	Assay Solution I (Component C)
TS	50 µL	Test Sample

NAD generation assay

1. Add 50 µL of cADPR standard, blank control, and test samples to solid black 96-well microplate (As shown in Table 1 and Table 2).

2. Add 50 µL/well of ADPRC working solution into each well of cADPR standard, blank control and test samples. *Note:* For a 384-well plate, add 12.5 µL of sample and 12.5 µL of ADPRC Reaction Mix Solution into each well.

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3. Incubate the reaction at room temperature for 60 minutes, protected from light.

NAD detection assay

1. Add 40 µL Quest Fluor™ NAD Probe (Component A) into each well of cADPR standard, blank control, and test samples (total of 140 µL/well), mix well.

2. Add 40 µL Assay Solution II (Component D) into each well (total of 180 µL/well), mix well.

   Note For a 384-well plate, add 10 µL of Quest Fluor™ NAD Probe and 10 µL Assay Solution II into each well.

3. Incubate the reaction at room temperature for 10 - 20 minutes, protected from light.

4. Add 30 µL Enhancer Solution (Component E) to each well to make the total NAD assay volume of 210 µL/well, and incubate at room temperature for 10-20 minutes, protected from light.

   Note For a 384-well plate, add 7.5 µL Enhancer Solution.

5. Monitor the fluorescence increase with a fluorescence plate reader at 420/480 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate cADPR samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

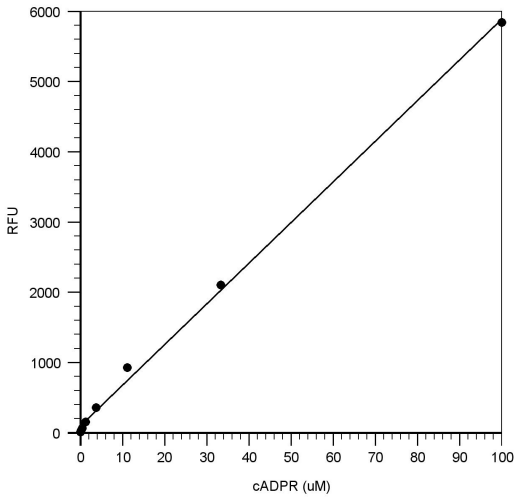

Figure 1. The concentration of cADPR was detected using Amplite™ Fluorimetric cADP-Ribose Assay Kit. Different concentrations of cADPR were incubated with ADPRC reaction mix for 20 min at RT before NAD detection reagent was added. The lowest detected concentration of cADPR is 100 nM.

DISCLAIMER