Technical Bulletin

Fluorimetric cADP-Ribose Assay Kit

Catalogue number MAK553

Product Description

cADP-ribose (cADPR) is a 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 by the NAD probe. This makes monitoring cADPR in tissues and cell cultures possible in the low nM range. The NAD+ detection using NAD probe is specific to NAD+ and has no reaction to NADH. This assay can be performed in a convenient 96-well or 384-well microplate.

Components

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

- NAD Probe 5 mL
  Catalogue Number MAK553A
- ADPRC Enzyme Mix 1 Vial
  Catalogue Number MAK553B
- Assay Solution I 12 mL
  Catalogue Number MAK553C
- Assay Solution II 5 mL
  Catalogue Number MAK553D
- Enhancer Solution 3.5 mL
  Catalogue Number MAK553E
- cADPR Standard 1 Vial
  Catalogue Number MAK553F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Fluorescence multiwell plate reader.
- Black, flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of cADPR Standards

1. Add 10 µL of purified water into the vial of cADPR Standard and mix well to produce a 5 mM stock solution.

Note: Store the unused cADPR standard stock solution at -20 °C in single use aliquots.

2. Prepare cADPR standard serial dilutions in Assay Solution I (ST1 – ST7) as shown in Table 1.

Table 1.
Serial dilution of cADPR Standards.

Dilution	cADPR Standard Vol (µL)	Serial Dilution Source	Assay Buffer Vol (µL)	Conc (µM)
ST1	4.5	From 5 mM Stock	220.5	100
ST2	75	From ST1	150	33.33
ST3	75	From ST2	150	11.11
ST4	75	From ST3	150	3.70
ST5	75	From ST4	150	1.23
ST6	75	From ST5	150	0.41
ST7	75	From ST6	150	0.13

Preparation of ADPRC Working Solution

Add 50 µL purified water into the vial of ADPRC Enzyme Mix and mix well. Transfer the whole content into 5 mL of Assay Solution I and mix well.

Note: ADPRC working solution is unstable, use it promptly.

Assay Reaction

NAD Generation:

1. Add 50 µL of cADPR standard, blank control, and test samples to solid black 96-well microplate. Use Assay Solution I for the blank.
2. Add 50 µL/well of ADPRC Working Solution into each well of cADPR standard, blank control, and test sample.

   Note: For 384-well plates, use 12.5 µL.
3. Incubate the reaction mixture at room temperature for 60 minutes, protected from light.

NAD Detection:

1. Add 40 µL NAD Probe into each well of cADPR standard, blank control, and test sample (total of 140 µL/well), mix well.
2. Add 40 µL Assay Solution II into each well (total of 180 µL/well), mix well.

   Note: For 384-well plates, use 10 µL of Probe and Assay Solution II.
3. Incubate the reaction at room temperature for 10 - 20 minutes, protected from light.
4. Add 30 µL Enhancer Solution 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 384-well plates, use 7.5 µL of Enhancer Solution.

Measurement

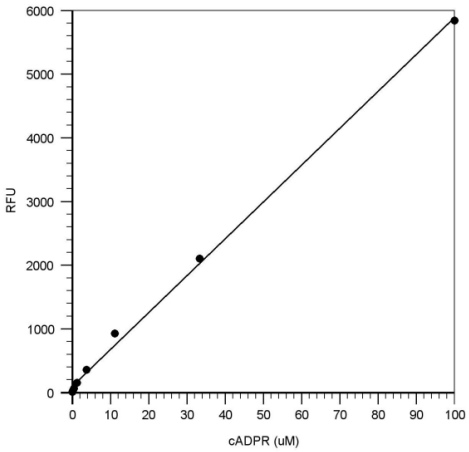
Monitor the fluorescence increase with a fluorescence plate reader at 420/480 nm.

Results

1. The reading (RFU) obtained from the blank standard well is used as a negative control.
2. Subtract the blank value from the other standards readings to obtain the base line corrected values.
3. Plot the standards readings to obtain the standard curve.
4. The concentration of cADPR in the test samples may be determined from the standard curve.

Figure 1

Typical cADPR Standard Curve



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