Elite™ cADP-ribose Assay Kit (Fluorescence)

CATALOG NUMBER: CA-CADP10, 100 assays

Description

cADP-ribose (cADPR) is a novel Ca^{2+} 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 NAD Fluor Dye. This makes monitoring cADPR in tissues and cell cultures possible in the low nM range.

The NAD^+ detection using NAD Fluor Dye is specific to NAD^+ and has no reaction to NADH. The fluorescent signal can be readily detected at Ex/Em = 420/480 nm. This assay can be performed in a convenient 96-well or 384-well microtiter plate.

Kit Components

- **Component A**: NAD Fluor Dye — 1 bottle (5 ml)
- **Component B**: ADPRC Enzyme Mix — 1 vial
- **Component C**: Assay Buffer I — 1 bottle (12 ml)
- **Component D**: Assay Buffer II — 1 bottle (5 ml)
- **Component E**: Enhancer Solution — 1 bottle (3.5 ml)
- **Component F**: cADPR Standard — 1 vial

Storage

Keep in freezer (-20 °C) and avoid exposure to light.

Shelf Life

All reagents are stable for at least 6 months after receipt when stored properly at the recommended conditions.

Materials required (but not supplied)

- 96 microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader.

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Assay Protocol (for 96-Well Plate)

Summary of the Assay

Add 50 µl cADPR standards or test samples (each well)

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Add 50 µl ADPRC working solution (each well)

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Incubate at room temperature (or 25 °C) for 60 minutes

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Add 40 µl NAD Fluor Dye (each well)

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Add 40 µl Assay Buffer II (each well)

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Incubate at room temperature (or 25 °C) for 10-20 minutes

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Add 30 µl Enhancer Solution

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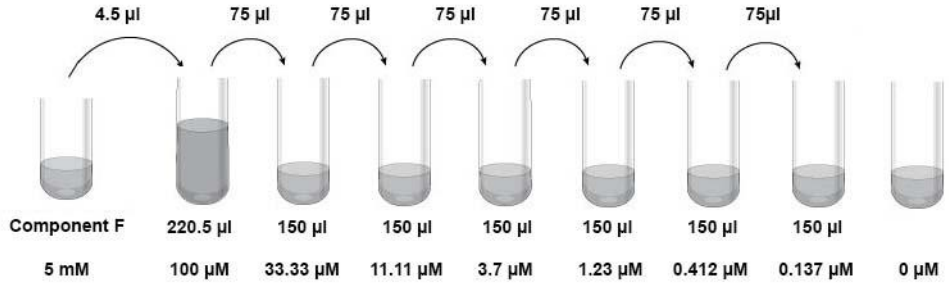
Incubate at room temperature (or 25 °C) for 10-20 minutes

↓

Monitor fluorescence intensity at Ex/Em = 420/480 nm

1. **Preparation of cADPR standard stock solution (5 mM):**
   Add 10 µl of ddH_2O into the vial of cADPR Standard (**Component F**) to make 5 mM (5 nmol/µl) cADPR standard stock solution.
   Note: The unused 5 mM cADPR standard stock solution should be stored at -20 °C.

2. **Preparation of cADPR standard solutions:**
   Add 4.5 µl of cADPR standard stock solution (5 mM) into 220.5 µl of Assay Buffer I (**Component C**) to generate 100 µM cADPR standard solution. Serial dilutions (1:3) will be made by Assay Buffer I as follows.



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3. Preparation of ADPRC working solution:

Add 50 µl of ddH_2O into the vial of ADPRC Enzyme Mix (**Component B**) and mix well. Transfer whole content into 5 ml of Assay Buffer I (**Component C**) and mix them well.

Note: ADPRC working solution is unstable, prepare it before use.

4. Sample experimental design:

Table 1. Layout of cADPR standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	…							
ST 1	ST 1	…	…								
ST 2	ST 2										
ST 3	ST 3										
ST 4	ST 4										
ST 5	ST 5										
ST 6	ST 6										
ST 7	ST 7										

Note: ST = cADPR Standard (ST1-ST7); BL=Blank Control; TS=Test Samples.

Table 2. Reagent composition for each well

cADPR Standards	Blank Control	Test Sample
Serial Dilutions: 50 µl	Assay Buffer I: 50 µl	50 µl

Note: Add the serially diluted cADPR standards from 0.137 µM to 100 µM into wells from ST 1 to ST 7 in duplicate.

5. Run the NAD assay:

NAD generation assay:

5.1. 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).

5.2. 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 mixture into each well.

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

NAD generation assay:

5.4. Add 40 µl NAD Fluor Dye (**Component A**) into each well of cADPR standard, blank control, and test samples (total of 140 µl/well), mix well.

5.5. Add 40 µl Assay Buffer II (**Component D**) into each well (total of 180 µl/well), mix well.

Note: For a 384-well plate, add 10 µl of NAD Fluor Dye and 10 µl Assay Buffer II into each well.

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

5.7. 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 10-20minutes, protected from light.

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

5.8. Monitor the fluorescence increase at Ex/Em = 420/480 nm using a fluorescence plate reader.

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Data Analysis

The reading (RFU) obtained from the blank standard well is used as a negativecontrol. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standardcurve and equation. This equation can be used to calculate cADPR samples.

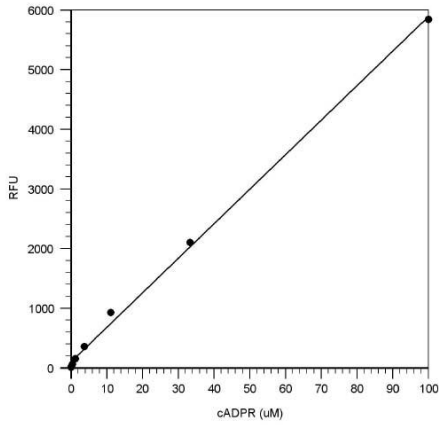

Figure 1. The concentration of cADPR was detected using Elite™ cADP-ribose Assay Kit. Different concentrations of cADPR were incubated with ADPRC reaction mix for 20 min at RT before NAD detection reagent was added. The lowest detected concentration of cADPR is 100 nM.

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