

TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

FITC-NAD (6-fluorescein-17-nicotinamide-adenine-dinucleotide)

Catalog #: 4673-500-01

Size: 250 µl

Description: FITC-NAD (figure 1) provides a convenient, non-isotopic alternative to radiolabeled NAD for use with enzymes requiring NAD as a substrate or cofactor. A number of proteins, including poly (ADP-ribose) polymerases (e.g. PARP-1, PARP-2), and the SIR2 family of NAD(+)-dependent histone/protein deacetylases, use NAD as a substrate for their function. FITC-conjugated NAD permits the direct measurement of PARP and other NAD-dependent enzymes by fluorescence microscopy or by incorporation of fluorescent-labeled poly (ADP-ribose) onto histones attached to a 96 well plate. FITC-NAD enters cells with the aid of Trevigen's Cell Permeabilization Solution (cat# 4674-250-01).

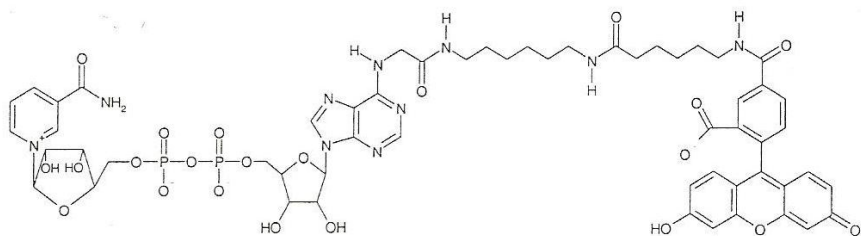


Figure 1: Structure of FITC-NAD

Physical State: Provided in solution at a concentration of 250 µM in deionized water. There is a 1:1 stoichiometry for incorporation; one FITC label for each NAD molecule.

Purity: > 98% by HPLC; > 95% by ³²P NMR.

Extinction Coefficient: 21,000 at 265 nm.

Storage: Store at -80 °C.

Applications:*

- ◆ Activity measurements of NAD-requiring enzymes.
- ◆ Assays to identify inhibitors of activators of NAD-requiring enzymes.

*Cell Permeabilization Solution (cat# 4674-250-01), is required for in-cell assays, and is needed for FITC-NAD entry.

Recommended Working Concentration: A working concentration of 5 µM has been effective in both cell staining and assays used to determine PARP activity; the working concentration, however, may require prior optimization based on applications or cell types used.

In Cell Assay:

1. Culture cells in media as recommended by supplier, and allow at least two passages prior to assay. Adherent cells may also be cultured in chamber slides.
2. Treat cells as desired; it is recommended to treat one sample with 2 mM 3-amino-benzamide as a control. Some treatments may require cells be incubated in serum-free medium prior to treatment. Allow at least 30 minutes to prepare reaction cocktail.
3. Calculate the volume of FITC-NAD reaction cocktail needed:

$$\text{Needed} = \frac{400 \mu\text{l solution}}{1.0 \times 10^6 \text{ cells}} \times \frac{\text{# cells total}}{\text{# cells total}} = \text{_____} \mu\text{l (X)}$$

4. Prepare Reaction Cocktail (method 1):

Sterile, deionized water	=	X • 0.74 = _____ µl
20X PARP Buffer	=	X • 0.05 = _____ µl
100 mM DTT	=	X • 0.01 = _____ µl
Cell Permeabilization Solution	=	X • 0.10 = _____ µl
NAD-FITC	=	X • 0.10 = _____ µl

Store reaction cocktail in the dark for at least 10 minutes prior to adding to cells.

(method 2):

Mix one part FITC-NAD, undiluted, with one part Cell Permeabilization Solution, undiluted, and incubate in the dark for at least 10 minutes prior to the addition of the remaining ingredients.

5. Wash cells twice with PBS, and add 200 µL of Reaction Cocktail per 5.0 x 10⁵ cells. Incubate cells at 37°C for one hour.
6. Discard Reaction Cocktail, and incubate cells in chilled 95% Ethanol (-20 °C) for 10 minutes. This fixes cells.
7. Discard 95% ethanol, and incubate cells in chilled 10% TCA (4 °C) for 15 minutes. This inactivates any PAR glycosylases.
8. Wash cells with PBS, mount with fluorescent mounting media, and view under fluorescent microscope.

Reference:

1. Bakondi E, Bai P, Szabó E E, Hunyadi J, Gergely P, Szabó C, Virág L. (2002) Detection of poly(ADP-ribose) polymerase activation in oxidatively stressed cells and tissues using biotinylated NAD substrate. J Histochem Cytochem 50:91-8.

TREVIGEN®

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