

# TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

## Fluorescein-NAD (6-fluorescein-17-nicotinamide-adenine-dinucleotide)

Catalog #: 4673-500-01

Size: 250 µl

**Description:** Fluorescein-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. Fluorescein-conjugated NAD permits the direct measurement of PARP and other NAD-dependent enzymes by fluorescence microscopy.

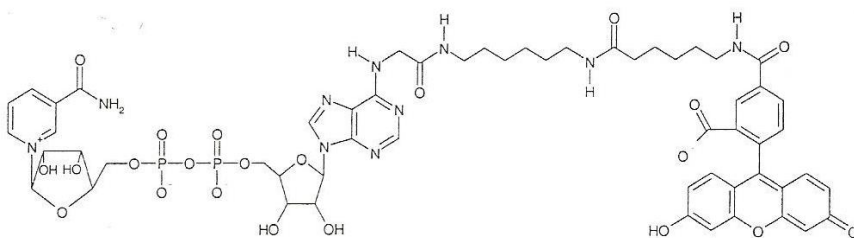


Figure 1: Structure of Fluorescein-NAD

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

**Extinction Coefficient:** 38,000 at 262 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 Fluorescein-NAD entry.

**Recommended Working Concentration:** A working concentration of 5-25 µM has been effective in cell staining to determine PARP activity; the working concentration, however, may require prior optimization based on application, cell type, or cell number used.

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## TREVIGEN®

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### In Cell Assay:

1. Culture cells in medium 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. Prepare 400  $\mu$ l of Fluorescein-NAD reaction cocktail per  $1.0 \times 10^6$  cells. Store reaction cocktail in the dark for at least 10 minutes prior to adding to cells in Step 4.

Reaction Cocktail for 25  $\mu$ M Fluorescein-NAD reaction per 400  $\mu$ l:

Sterile, deionized water	296 $\mu$ l
20X PARP Buffer	20 $\mu$ l
100 mM DTT	4 $\mu$ l
Cell Penetrating Peptide Solution	40 $\mu$ l
250 $\mu$ M NAD-Fluorescein	<u>40 <math>\mu</math>l</u>
	400 $\mu$ l

\* Fluorescein NAD is a highly charged molecule and will not enter live cells without the aid of a cell penetrating peptide. Please see Morris et al<sup>2</sup> for further information.

**Note:** Reaction Mix can also be prepared by first mixing one part Fluorescein-NAD with one part Cell Permeabilization Solution and incubate in the dark for at least 10 minutes prior to the addition of the remaining ingredients.

4. Wash cells twice with PBS, and add 200  $\mu$ l of Reaction Cocktail per  $5.0 \times 10^5$  cells. Incubate cells at 37°C for one hour.
5. Discard Reaction Cocktail, and incubate cells in chilled 95% Ethanol (-20°C) for 10 minutes to fix cells.
6. Discard 95% ethanol, and incubate cells in chilled 10% TCA (4°C) for 15 minutes to inactivate PAR glycosylases.
7. Wash cells with PBS, mount with fluorescent mounting medium, 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.
2. Morris, M.C., Gros, E., Aldrian-Herrada, G., Choob, M., Archdeacon, J., Heitz, F., and Divita, G. (2007). A non-covalent peptide-based carrier for in vivo delivery of DNA mimics. Nucleic Acids Res 35, e49.

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