DATENBLATT

Fluorescein-NAD
(6-fluorescein-17-nicotinamide-dinucleotide)
Cat.# 4673-500-01

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. Fluorescein-NAD enters cells with the aid of Trevigen's Cell Permeabilization Solution (cat# 4674-250-01).

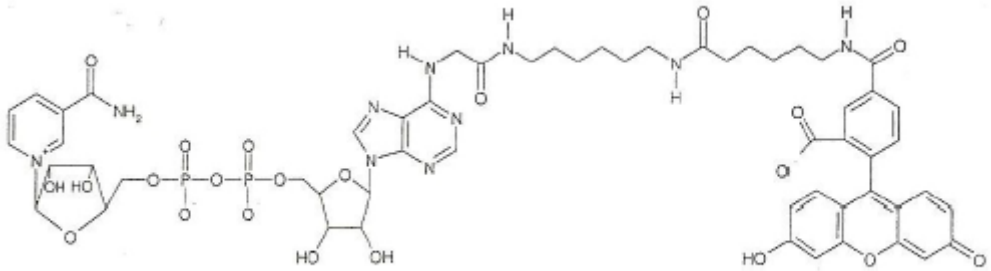

Figure 1: Structure of Fluorescein-NAD

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.

Gentaur Molecular Products
Marienbongard 20
52062 Aachen Deutschland

*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.

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-aminobenzamide as a control. Some treatments may require cells be incubated in serumfree medium prior to treatment. Allow at least 30 minutes to prepare reaction cocktail.
3. Prepare 400 µl of Fluorescein-NAD reaction cocktail per 1.0 x 106 cells. Store reaction cocktail in the dark for at least 10 minutes prior to adding to cells in Step 4.

Reaction Cocktail for 25 µM Fluorescein-NAD reaction per 400 µl:

Sterile, deionized water	296 µl
20X PARP Buffer	20 µl
100 mM DTT	4 µl
Cell Permeabilization Solution	40 µl
250 µM NAD-Fluorescein	40 µl
	400 µl

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 µl of Reaction Cocktail per 5.0 x 105 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.