TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Cell Permeabilization Solution

Catalog #: **4674-250-01** **Size: 250 µl**

Description: Cell Permeabilization Solution allows for the efficient transfer of Fluorescein-conjugated NAD across cellular membranes. The solution contains a selective cell permeable peptide to deliver Trevigen's Fluorescein-NAD (cat# 4673-500-01) from the outside to the inside of intact cells.

Physical State: Peptide provided as solution in deionized water.

Storage: Store at -20°C.

Applications:

- In-cell activity measurements of NAD-requiring enzymes.
- In-cell Assays to identify inhibitors of activators of NAD-requiring enzymes.

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. Prepare 400 µl of Fluorescein-NAD reaction cocktail per 1.0 x 10^6 cells. Store reaction cocktail in the dark for at least 10 minutes prior to adding cells.

   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.

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4. Wash cells twice with PBS, and add 200 µl of Reaction Cocktail per 5.0 x 10^5 cells. Incubate cells at 37°C for one hour.

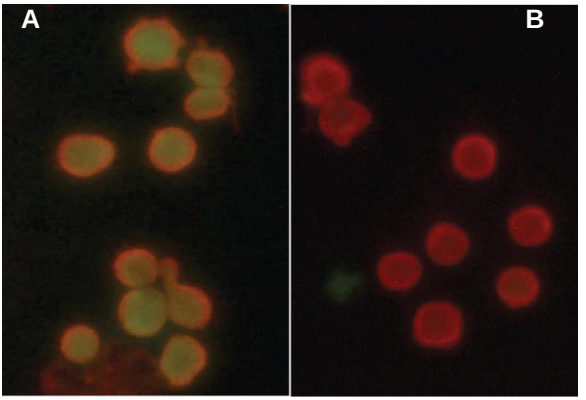
5. Discard Reaction Cocktail, and incubate cells in chilled 95% Ethanol (-20°C) for 10 minutes to fix cells.

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 media, and view under fluorescent microscope.

Reference:

1. Fischer, R., Fotin-Mleczek, M., Hufnagel, H. and Brock, R. (2005), Break on through to the Other Side—Biophysics and Cell Biology Shed Light on Cell-Penetrating Peptides. ChemBioChem, 6: 2126–2142.



Fluorescent Image of In-Cell Assay Results

Intracellular synthesis of fluorescein-labeled poly ribose from fluorescein-NAD by Poly (ADP-ribose) polymerase (PARP) in the presence of Evan's Blue is shown. Wehi (5 x 10^5 cells) were treated with 50 µM H_2O_2 under serum-free conditions for 5 minutes, incubated as described above for one hour at 37°C in the absence (panel A) or presence (panel B) of 2 mM 3-AB, and fixed in chilled EtOH. Cells incubated in the absence of cell permeabilization solution did not exhibit fluorescence (not shown).

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