ADP-ribose-pNP

Catalog number: 11700
Unit size: 1 mg

Component	Storage	Amount
ADP-ribose-pNP	Freeze (<-15 °C), Minimize light exposure	1 mg

OVERVIEW

ADP-ribose-pNP is a colorimetric substrate for assessing activity of poly(ADP-ribose)polymerase (PARP) enzymes. The absorbance of released p-nitrophenol is determined at 405 nm, and the slope of the calibration curve is used to convert the absorbencies to moles of product generated. With ADP-ribose-pNP as the colorimetric substrate, PARP-1 was determined to have the largest Km and Vmax values (151 uM and 1.30 nmolmin-1mg-1 respectively) followed by tankyrase-1 (82 uM and 18 pmolmin-1mg-1 respectively) and VPARP (46 uM and 2 pmolmin-1mg-1 respectively). This colorimetric substrate can be used to determine the kinetic parameters for PARP-1, tankyrase-1, and VPARP, and to screen small-molecule inhibitors of PARP-1, tankyrase-1, and VPARP. ADP-ribose-pNP-based continuous assay has considerable advantages over standard discontinuous PARP assays, enabling the high throughput screening of PARP-1, tankyrase-1, and VPARP activities and their inhibitors.

AT A GLANCE

Important The following recommended procedure can be adapted for measuring PARP-1, tankyrase-1, and VPARP activities and their inhibitors. The optimum conditions must be determined experimentally for each test.

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	405 nm
Recommended plate:	Solid white

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. ADP-ribose-pNP stock solution:
Make 5 - 10 mM stock solution in H_2O.

Note The stock solution should be used promptly.

PREPARATION OF WORKING SOLUTION

ADP-ribose-pNP working solution:
Prepare 0.25 mM assay solution by diluting the stock solution with assay buffer (50mM Tris, 10mM $MgCl_2$, pH 8.0).

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 0.01 mL/well of sample solution into 0.09 mL/well assay solution to make a final volume of 0.1 mL in a 96-well clear plate.

2. Monitor the plate using an absorbance microplate reader at 405 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

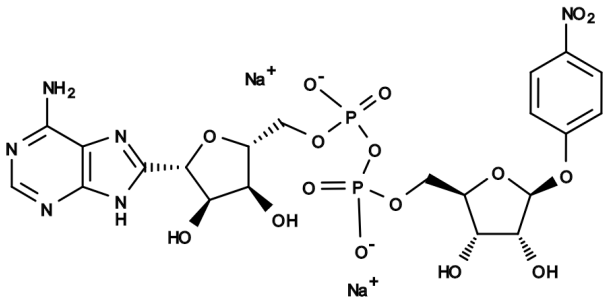

Figure 1. Chemical structure for ADP-ribose-pNP

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