INSTRUCTIONS



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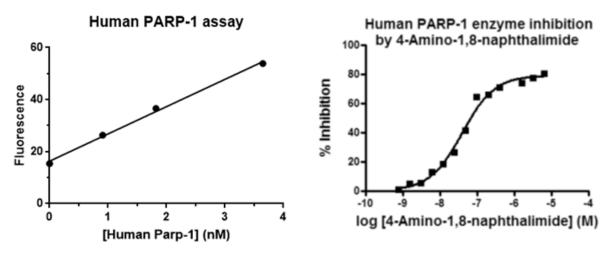
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ProFoldin Human Poly (ADP-ribose) Polymerase-1 Assay Kits

Human Poly (ADP-ribose) Polymerase-1 Assay Kit Human Poly (ADP-ribose) Polymerase-1 Assay Kit Plus Catalog No. PAR100K Catalog No. PAR100KE

Introduction

Poly (ADP-ribose) polymerase-1 (PARP-1) responses to DNA damage and synthesizes poly ADP-ribose (PAR) that is transferred to itself and a variety of acceptor proteins. Excessive activation of PARP-1 not only depletes NAD⁺ but also generates toxic PAR that lead to cell death. PARP-1 is an anti-cancer drug target. It is also a potential drug target for other diseases such as stroke, ischemia and reperfusion where high-level DNA damage occurs. The human PARP-1 assay is based on measurement of its product PAR (poly ADP-ribose) that binds to a fluorescent dye and enhances its fluorescent signal.



The **Human Poly (ADP-ribose) Polymerase-1 Assay Kit** (catalog number PAR100K) includes 800 μ l of 10 x the assay buffer, 55 μ l of 100x NAD⁺, 55 μ l of 100x DNA and 220 μ l of 100 x fluorescence dye for 100 assays of human PARP-1 in a 96-well plate format. The kit does not include Human PARP-1.

The Human Poly (ADP-ribose) Polymerase-1 Assay Kit Plus (catalog number PAR100KE) includes 800 μ l of 10 x the assay buffer, 55 μ l of 200x NAD⁺, 55 μ l of 100x DNA, 17 μ l of 300 x Human PARP-1 and 220 μ l of 100x fluorescence dye for 100 assays of human PARP-1 in a 96-well plate format.

Assay Protocol

1. Reagent preparation:

10 x DNA: dilute the 100 x DNA 10 -fold with water

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10 x enzyme: dilute the 400 x Human PARP-1 40 –fold with 1 x Buffer

1 mM NAD⁺: dilute the 10 mM stock 10 -fold with water

1 x fluorescence dye: dilute the 100 x fluorescence dye 100-fold with water

2. Reaction:

The total volume of each reaction mixture is 50 μ l including: 30 μ l of H₂O, 5 μ l of 10 x buffer, 5 μ l of 10 x DNA, 5 μ l of 10 x human PARP-1, 5 μ l of 1 mM NAD⁺. Incubate the reaction mixture at 37°C for 60 min.

3. **Detection**:

Add 200 μ l of the 1 x fluorescence dye into the 50 μ l of the reaction mixture. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm.

Assay Protocol for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at http://www.profoldin.com/services.html.

Related Products

HDPA100K	Human DNA Polymerase Alpha Assay Kit
HDPA100KE	Human DNA Polymerase Alpha Assay Kit Plus
DPA100KE	E. coli DNA Polymerase III Alpha Assay Kit Plus
DPA100KH	H. influenzae DNA polymerase Assay Kit Plus
DPA100KN	S. pneumoniae DNA polymerase Assay Kit Plus
DPB100KE	Human DNA Polymerase Beta Assay Kit Plus
DPG100K	Human DNA Polymerase Gamma Assay Kit
RPA100KE	E. coli RNA Polymerase Assay Kit Plus
T7RPA100K	T7 RNA Polymerase Assay Kit
MRPA100K	Human Mitochondrial RNA Polymerase Assay Kit
MRPA100KE	Human Mitochondrial RNA Polymerase Assay Kit Plus
RPA100KE	E. coli RNA Polymerase Assay Kit Plus
AMV100KE	AMV Reverse Transcriptase Assay Kit Plus
HIV100KE	HIV Reverse Transcriptase Assay Kit Plus
MLV100KE	M-MLV Reverse Transcriptase Assay Kit Plus

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.