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INSTRUCTIONS

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

Catalog Number **PAR100K**

Introduction

Poly (ADP-ribose) polymerase-1 (PARP-1) responds 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 that binds to a fluorescent dye and enhances its fluorescent signal. The Assay Kit (catalog number PAR100K) includes the assay buffer, DNA template and fluorescence dye for 100 assays of human PARP-1 in a 96-well plate format.

Assay Protocol

1. 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 (Buffer PA), 5 μ l of 10 x DNA, 5 μ l of 10 x human PARP-1, 5 μ l of 0.5 mM NAD⁺. Incubate the reaction mixture at room temperature for 45 min.

Note: Human PARP-1 is commercially available from Trevigen, catalog number 4668-100-01. The recommended final enzyme concentration in the reaction mixture is 250 U/ml.

2. 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.

Note: Fluorescence signals are sensitive to temperature changes. Please keep the temperature consistent during the measurement.