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Data Sheet

PARPtrap™ Assay Kit

Catalog # 80584

DESCRIPTION: The *PARPtrap™ Assay Kit* is designed to measure PARP1/DNA complex formation in a high throughput screening assay using fluorescence polarization (FP). PARP1 is known to bind damaged DNA through its N-terminal zinc finger domain. After PARP1 ribosylates itself (autoribosylation), it dissociates from DNA due to the accumulated negative charge of the ribosyl polymer. Trapped PARP-DNA complexes have been shown to be cytotoxic to cancer cells. The *PARPtrap™ Assay Kit* comes in a convenient 96-well format, with purified PARP1 enzyme, fluorescent labeled nicked DNA, and PARPtrap™ assay buffer for 100 enzyme reactions. The key to the *PARPtrap™ Assay Kit* is the fluorescent labeled nicked DNA. Without the PAR reaction, PARP1 binds to the fluorescent labeled nicked DNA, resulting in the emission of highly polarized light. However, after autoribosylation of PARP1, the nicked DNA is dissociated from PARP1 and rotates freely, emitting less polarized light (Fig. 1).

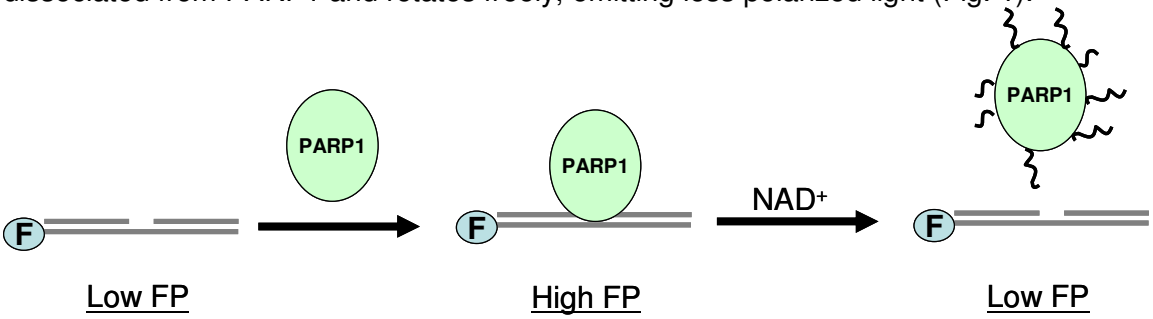

Figure 1. PARPtrap™ Assay Kit schematic

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
80501	PARP1, GST-tag	5 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
	50x Fluorescent labeled nicked DNA	0.1 ml	-80°C	
	5x PARPtrap™ assay buffer	1.5 ml	-80°C	
	10x NAD^+	0.5 ml	-80°C	
	Black 96-well plate	1	Room Temp.	
	Plate lid	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring fluorescence polarization
Adjustable micropipettor and sterile tips
Rotating or rocker platform

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APPLICATIONS: Great for screening small molecules that enhance PARP1/DNA trapping for drug discovery and HTS applications.
STABILITY: Up to 1 year when stored as recommended.

REFERENCES: Murai, J., *et al. Molecular Cancer Therapeutics* 2014. **13:**433-443.
Murai, J., *et. al. Cancer Research* 2012. **72:**5588-5599.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Prepare the master mixture (20 µl): N wells x (6 µl **5x PARPtrap™ assay buffer** + 1 µl **50x Fluorescent labeled nicked DNA** + 13 µl distilled water). Add 20 µl to every well.

2) To the well designated as "Blank", add 6 µl of **5x PARPtrap™ assay buffer** + 14 µl of distilled water.

3) Add 5 µl of inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 5 µl of the same solution without inhibitor (Inhibitor buffer). *Note: The PARPtrap™ Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in 10% DMSO aqueous solution and using 5 µl per PARP/DNA reaction.*

	Positive Control	Test Inhibitor	Negative Control	Blank
5x PARPtrap™ assay buffer	6 µl	6 µl	6 µl	6 µl
50x Fluorescent labeled nicked DNA	1 µl	1 µl	1 µl	-
Water	13 µl	13 µl	13 µl	14 µl
Test Inhibitor	–	5 µl	–	
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl	5 µl
1x PARPtrap™ assay buffer	–	–	20 µl	-
PARP1 (2.5 ng/µl)	20 µl	20 µl	–	20 µl
10x NAD^+	5 µl	5 µl	5 µl	5 µl
Total	50 µl	50 µl	50 µl	50 µl

4) Prepare **1x PARPtrap™ assay buffer** by diluting **5x PARPtrap™ assay buffer** with four parts distilled water. Dilute only enough required for the assay.

5) Thaw **PARP1** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **PARP1** required for the assay and dilute enzyme to 2.5 ng/µl with **1x PARPtrap™ assay buffer**. Aliquot remaining **PARP1** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **PARP1** enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

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6) To the wells designated as "Negative Control", add 20 µl of **1x PARPtrap™ assay buffer**.

7) Add 20 µl of diluted **PARP1** enzyme to the wells designated “Positive Control,” “Blank,” and "Test Inhibitor". Incubate at room temperature for 30 minutes.

8) Initiate **PARP1** enzymatic reaction by adding 5 µl of **10x NAD⁺** and incubate the plate for 45 min at room temperature.

9) Read the fluorescent polarization of the sample in a microtiter-plate reader capable of excitation at wavelengths ranging from 475-495 nm and detection of emitted light ranging from 518-538 nm. Blank value is subtracted from all other values.

CALCULATING RESULTS:

Definition of Fluorescence Polarization:

$$P = \frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}$$

where $I_{\parallel}$ = Intensity with polarizers parallel and $I_{\perp}$ = Intensity with polarizers perpendicular. Most instruments display fluorescence polarization in units of mP.

$$mP = \left(\frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}\right) x\ 1000$$

The equation above assumes that light is transmitted equally well through both parallel and perpendicular oriented polarizers. In practice, this is generally not true and a correction must be made to measure the absolute polarization state of the molecule. This correction factor is called the "G Factor".

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000 \qquad \textbf{OR} \qquad mP = \left(\frac{G(I_{II}) - I_{\perp}}{G(I_{II}) + I_{\perp}}\right) x\ 1000$$

The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

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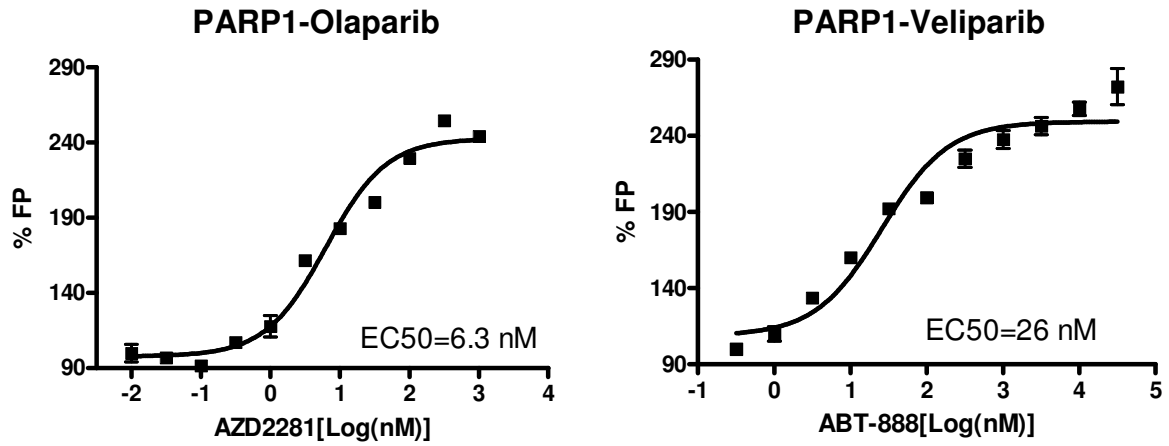
Example of Assay Results:



PARP1/DNA trapping measured in the presence of increasing concentrations of Olaparib (AZD2281) (left) or Veliparib (ABT-888) (right) using the *PARPtrap™ Assay Kit,* BPS Cat. #80584. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
PARP1 Chemiluminescent Assay Kit	80551	96 rxns
PARP1 Chemiluminescent Assay Kit	80569	384 rxns
PARP1 Colorimetric Assay Kit	80580	96 rxns
PARP2 Assay Kit	80552	96 rxns.
PARP3 Assay Kit	80553	96 rxns.
PARP5A (TNKS1) Assay Kit	80573	96 rxns.
PARP5B (TNKS2) Assay Kit	80579	96 rxns.
PARP6 Assay Kit	80556	32 rxns.
PARP1 Enzyme	80501	10 µg
PARP2 Enzyme	80502	10 µg
PARP3 Enzyme	80503	10 µg
PARP6 Enzyme	80506	10 µg
TNKS2 (PARP5A) Enzyme	80504	10 µg
TNKS2 (PARP5B/C) Enzyme	80505	10 µg
PARP7 Enzyme	80507	10 µg
PARP9 Enzyme	80509	10 µg
PARP11 Enzyme	80511	10 µg
PARP12 Enzyme	80512	10 µg