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Zuschläge

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Description

The PARPtrap™ Assay Kit for PARP1 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 DNA-binding domains. Binding to DNA activates PARP1 and in the presence of NAD⁺ PARP1 ribosylates itself (auto-ribosylation), what in consequence leads to PARP1 dissociation from the DNA due to the accumulated negative charge of the ribosyl polymer. In the presence of some inhibitors, however, PARP remains bound to the DNA, a phenomenon termed trapping. Trapped PARP-DNA complexes have been shown to be highly cytotoxic to cancer cells, therefore such inhibitors may be desirable for cancer treatment.

The PARPtrap™ Assay Kit for PARP1 comes in a convenient 96-well format, with purified PARP1 enzyme, fluorescent-labeled oligonucleotide duplex, and PARPtrap™ assay buffer for 100 enzyme reactions. The key to the PARPtrap™ Assay Kit for PARP1 is the fluorescent-labeled oligonucleotide duplex. In the absence of ribosylation, PARP1 binds to the fluorescent probe, forming a large complex and resulting in the emission of highly polarized light. However, after auto-ribosylation, PARP1 dissociates from the oligonucleotide duplex, which then rotates freely, emitting less polarized light (Fig. 1). Addition of a PARP1 inhibitor results in trapping of PARP1 to the fluorescent oligonucleotide duplex, and increases the FP signal in a dose dependent manner.

The PARPtrap™ Assay Kit for PARP1 is a fluorescence polarization homogeneous assay. The FP signal is measured using a fluorescent microplate reader *capable of measuring fluorescence polarization*.

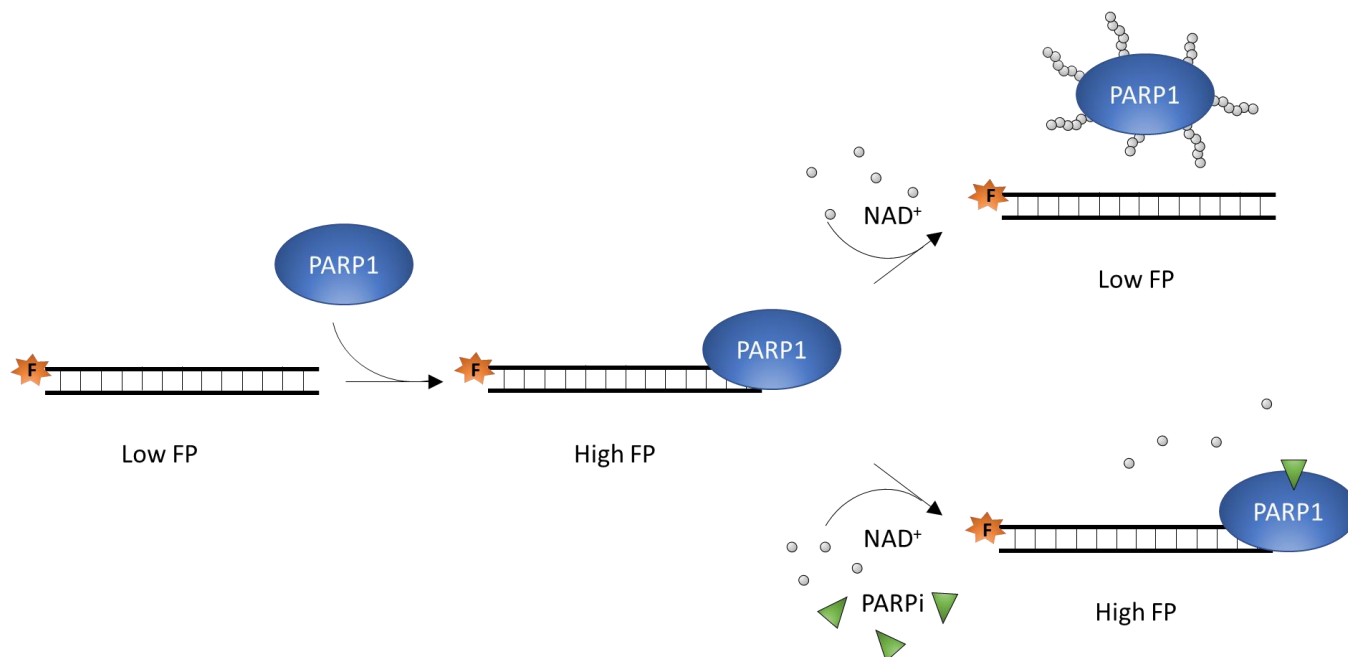


Figure 1. PARPtrap™ Assay Kit for PARP1 schematic

Applications

Great for screening small molecules that enhance PARP1/DNA trapping in drug discovery and HTS applications, and for determination of compound IC₅₀.

Supplied Materials

Catalog #	Name	Amount	Storage	
80501	PARP1, GST-tag *	1 µg	-80°C	<i>Avoid multiple freeze/thaw cycles</i>
78273	Fluorescent labeled oligonucleotide duplex (25 nM)	100 µl	-80°C	
	5x PARPtrap™ assay buffer	2 x 1 ml	-80°C	
	10x NAD+	500 µl	-80°C	
79685	Black 96-well plate		Room Temp	

* ***The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.***

Materials Required but Not Supplied

- Fluorescent microplate reader ***capable of measuring fluorescence polarization***
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to 1 year from date of receipt when the materials are stored as directed. ***Avoid multiple freeze/thaw cycles!***

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The PARPtrap™ Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in no higher than 10% DMSO aqueous solution and using 5 µl per well.

Assay Protocol

- All samples and controls should be performed in duplicate
- The assay should include a “Blank”, a “Reference”, a “Low FP control”, and a “High FP control”

Preparing Your Reagents

- 1) Prepare the Master Mix (20 µl per well): N wells x (6 µl of **5x PARPtrap™ assay buffer** + 1 µl of **25 nM Fluorescent labeled DNA** + 13 µl of distilled water).
For example, for 20 wells prepare: 120 µl of 5x PARPtrap™ assay buffer + 20 µl of 25 nM Fluorescent labeled DNA + 260 µl of distilled water.
- 2) Prepare **1x PARPtrap™ assay buffer** by diluting one part of **5x PARPtrap™ assay buffer** with four parts of distilled water. Dilute only enough required for the assay.

- 3) Prepare the inhibitor solution.
If the inhibitor compound is dissolved in DMSO, make a 10-fold dilution in water so the final concentration of DMSO will be 1% in all samples. If the compound was dissolved in a diluent other than DMSO, use that diluent to prepare the control diluent solution.
- 4) Prepare the enzyme:
Thaw **PARP1** enzyme on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Dilute the enzyme to ~0.5 ng/μl with **1x PARPtrap™ assay buffer**.
Note: **PARP1** enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Although we do not recommend it, if not using all the wells of the assay at once calculate the amount of PARP1 required for the assay, dilute only the amount sufficient for the assay and aliquot the remaining undiluted **PARP1** enzyme. Store single use aliquots at -80°C. Do not re-use a thawed aliquot or diluted enzyme.
- 5) Add each component of the reaction to the “High FP control”, “Low FP control”, “Test inhibitor” and “Reference” wells following the table below:

	High FP Control	Low FP Control	Test Inhibitor	Reference
Master Mix	20 μl	20 μl	20 μl	20 μl
Test Inhibitor	-	-	5 μl	-
Diluent solution (no inhibitor)	5 μl	5 μl	-	5 μl
1x PARPtrap™ assay buffer	-	-	-	20 μl
PARP1 (0.5 ng/μl) as prepared in step 3	20 μl	20 μl	20 μl	-
Total	45 μl	45 μl	45 μl	45 μl

- 6) To the wells designated as “Blank”, add 12 μl of **5x PARPtrap™ assay buffer** + 28 μl of distilled water + 5 μl of diluent (for example DMSO 10%).
Note that the “Blank” contains buffer components of the reaction but does not contain the fluorescent probe or PARP1.

	Blank
5x PARPtrap™ assay buffer	12 μl
Water	28 μl
Diluent solution (no inhibitor)	5 μl
Total	45 μl

Notes:

- Reference: a negative control lacking PARP1, in which all the fluorescent DNA is present in free form and FP is lowest (*this is different from the Blank in which there is no fluorescent probe*). **The reference is needed to perform calculations.**

- High FP control: a condition in which no NAD⁺ will be added, therefore all of PARP1 that is bound to fluorescent DNA cannot undergo ribosylation and remains associated to the DNA, resulting in the maximum FP allowed by the kit.
- Low FP control: a condition in which all of PARP1 that is bound to the fluorescent DNA is allowed to undergo ribosylation in the presence of NAD⁺ and dissociates from the DNA, resulting in mostly free DNA and low FP. This is the condition corresponding to maximum PARP1 ribosylation activity, in the absence of inhibitor.
- Test inhibitor: FP will increase in proportion to the level of inhibitory activity and resultant trapping onto the fluorescent nucleotide duplex.
- Diluent solution: same concentration of DMSO as used to prepare the test inhibitor, but without inhibitor

7) Incubate at room temperature for 30-60 minutes.

8) To the wells indicated as “High FP control”, add 5 µl distilled water.

9) Initiate PARP1 enzymatic reaction by adding 5 µl of **10x NAD⁺** to all other wells, including “Blank”, “Low FP control”, “Test inhibitor”, and “Reference”.. **Do not add NAD⁺ to the “High FP control”.**

This brings the final reaction volume to 50 µl.

Incubate the plate for 60 min at room temperature

10) Read the fluorescent polarization of the sample in a microtiter-plate reader capable of excitation at wavelengths ranging from 470-480 nm and detection of emitted light ranging from 508-528 nm. Blank value is subtracted from all other values.

Example Results

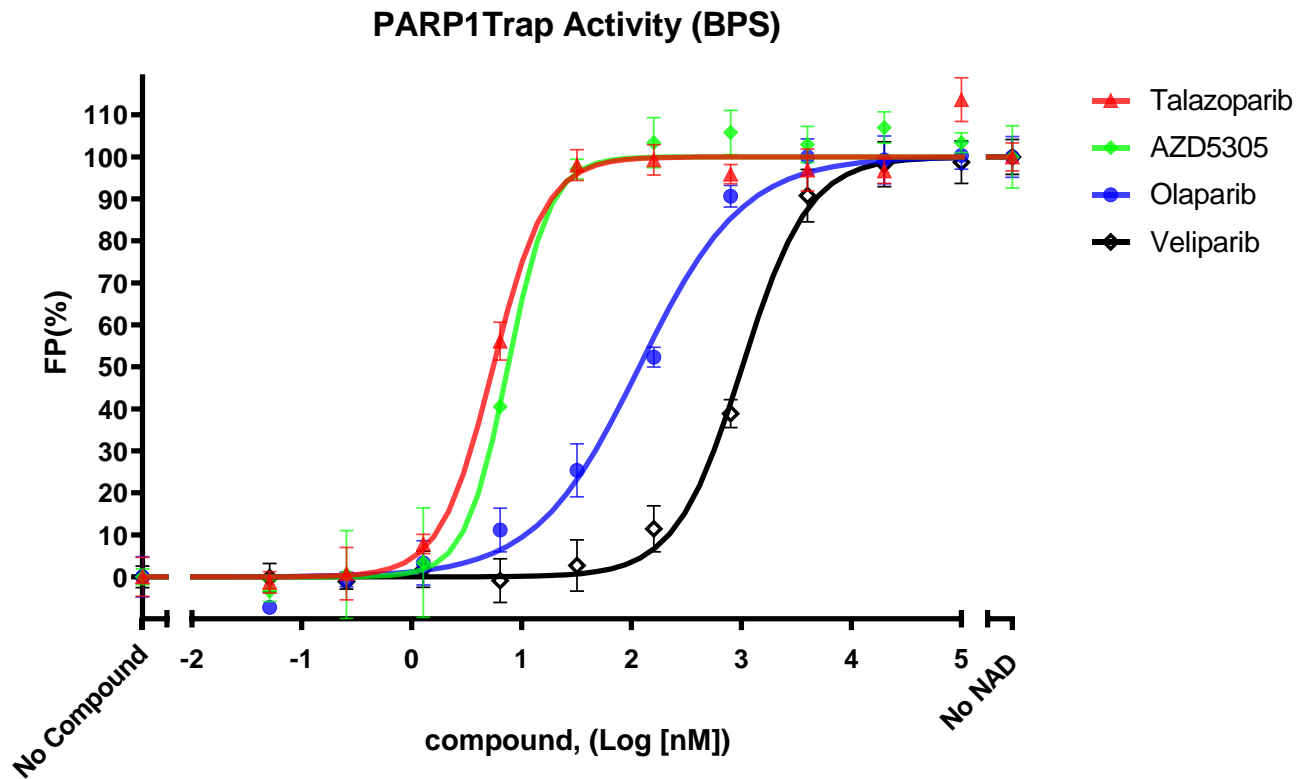


Figure 1: PARP1/DNA trapping measured in the presence of increasing concentrations of Talazoparib (Selleckchem), Olaparib (LC Laboratories), Veliparib (Selleckchem) and AZD5305 (MedChemExpress) using the PARPtrap™ Assay Kit, BPS Cat. #80584. “No compound” corresponds to the “Low FP control” and “no NAD” corresponds to the “High FP control”.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

CALCULATING RESULTS:**Definition of Fluorescence Polarization:**

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

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

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

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".

$$mP = \left(\frac{I_{\parallel} - G(I_{\perp})}{I_{\parallel} + G(I_{\perp})} \right) \times 1000 \quad \text{OR} \quad mP = \left(\frac{G(I_{\parallel}) - I_{\perp}}{G(I_{\parallel}) + I_{\perp}} \right) \times 1000$$

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 how to establish the G-factor.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

1. Murai, J. *et al.* Molecular Cancer Therapeutics 2014. 13: 433-443
2. Murai, J. *et al.* Cancer Research 2012. 72: 5588-5599
3. Zandarashvili, L. *et al.* Science 2020. 368(6486): 6367

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
PARPtrap™ Assay Kit for PARP1	80584	96 & 384 rxns
PARPtrap™ Assay Kit for PARP2	78296	96 & 384 rxns
PARPtrap™ Combo Assay Kit for PARP1 and PARP2	78317	384 rxns
Set of PARP inhibitors	78318	8 x 50 µl
Fluorescent labeled oligonucleotide duplex (25 nM)	78273	100 µl
Fluorescent labeled nicked oligonucleotide duplex (12.5 nM)	78297	100 µl
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