Description

The PARPtrap™ Combo Assay Kit for PARP1 and PARP2 is a kit designed to measure DNA complex formations of PARP1 and PARP2 enzymes in a high throughput screening assay using fluorescence polarization (FP). PARP1 and PARP2 enzymes are known to bind damaged DNA through their DNA-binding domains. Binding to DNA activates these enzymes and in the presence of NAD+, PARP1 and PARP2 ribosylate themself (autoribosylation), leading to their dissociation from the DNA due to the accumulated negative charge of the ribosyl polymer. In the presence of some inhibitors, however, these PARPs remain 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™ Combo Assay Kit for PARP1 and PARP2 comes in a convenient 384-well format, with purified PARP1 and PARP2 enzymes, fluorescent-labeled DNA probes, and PARPtrap™ assay buffers sufficient to perform 200 reactions for each PARP enzyme, respectively. The key to the PARPtrap™ Combo Assay Kit are the fluorescent-labeled DNA probes recognized by PARP1 and PARP2. In the absence of ribosylation, PARP1/2 binds to the DNA fluorescent probe, forming a large complex and resulting in the emission of highly polarized light. However, after autoribosylation, PARP1/2 dissociates from the oligonucleotide duplex, which then rotates freely, emitting less polarized light (Fig. 1). Addition of a PARP inhibitor results in trapping of PARP1/2 to the fluorescent-labeled DNA, and increases the FP signal in a dose dependent manner.

The combo kit is particularly useful to directly and specifically compare, in a single assay, the effect and potency of a compound on PARP1 and PARP2.

The PARPtrap™ Combo Assay Kit for PARP1 and PARP2 is a homogeneous fluorescence polarization assay. The FP signal is measured using a fluorescent microplate reader *capable of measuring fluorescence polarization.*

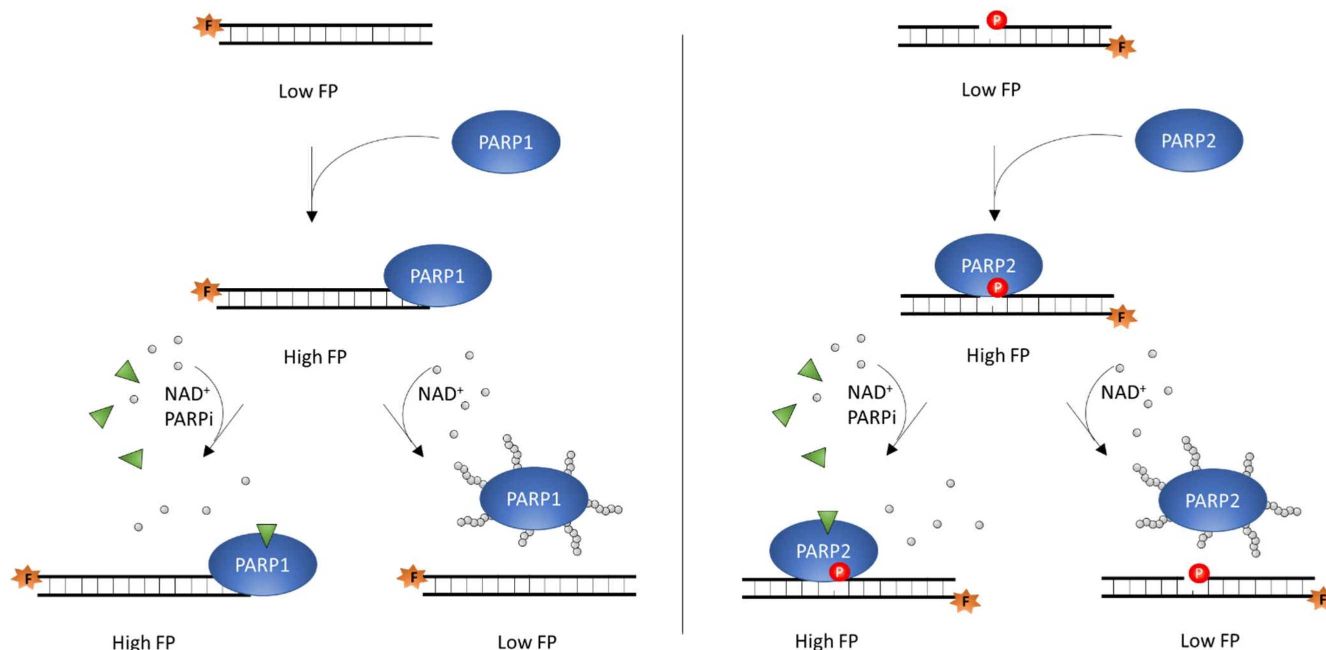

Figure 1. PARPtrap™ Combo Assay Kit for PARP1 and PARP2 schematic

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Applications

Great for screening small molecules that enhance PARP1/2 trapping on DNA in drug discovery and HTS applications, and for determination of compound IC_{50}.

Allows direct comparison of drug effects on PARP1 and PARP2.

Supplied Materials

	Catalog #	Name	Amount	Storage	
PARP1 specific reagents	80501	PARP1, GST-tag *	1 µg	-80°C	***Avoid multiple freeze/ thaw cycles***
	78273	Fluorescent labeled oligonucleotide duplex (25 nM)**	100 µl	-80°C	
		5x PARPtrap™ assay buffer	2 x 1 ml	-80°C	
PARP2 specific reagents	80502	PARP2, GST-tag *	8 µg	-80°C	
	78297	Fluorescent labeled nicked oligonucleotide duplex (100 nM)**	12.5 µl	-80°C	
		5x PARPtrap™ assay buffer 2	2 x 1 ml	-80°C	
		10x NAD+	2 x 500 µl	-80°C	
	79961	Black 384-well plate		Room Temp	

**** The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.***
*****Note that each PARP assay protocol uses its own fluorescent DNA substrate***

Materials Required but Not Supplied

- DTT
- 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™ Combo 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 2.5 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:
When using PARP1 and PARP2 in parallel:
- The 1x assay buffer used with PARP2 contains DTT whereas the assay buffer used with PARP1 does not. Prepare the 1x assay buffer for each enzyme according to directions using the corresponding 5x assay buffer.
- The buffers and the fluorescent DNA substrates differ between PARP1 and PARP2, therefore controls must be added using the corresponding components. Thus, an assay measuring both PARP1 and PARP2 activity in parallel should contain two sets of “Low FP”, “High FP”, “Reference” and “Blank”, meaning one set of controls with PARP1 assay components and the other set of controls with PARP2 assay components.

<u>For reactions containing PARP1 enzyme</u>

1) Prepare the Master Mix 1 (10 µl per well): N wells x (3 µl of **5x PARPtrap™ assay buffer** + 0.5 µl of **25 nM Fluorescent labeled DNA** + 6.5 µl of distilled water).
   For example, for 40 wells prepare: 120 µl of 5x PARPtrap™ assay buffer + 20 µl of 25 nM Fluorescent labeled DNA + 260 µl of distilled water.

   Note: the fluorescent labeled DNA has been optimized for PARP1 assay. Do not use the fluorescent nicked DNA to measure PARP1 trapping.

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 test inhibitor solution.
   If the test inhibitor compound is dissolved in DMSO, make a 10-fold dilution in water to make a 10% DMSO (aqueous) solution.
   Prepare the control diluent solution: for the wells labeled “High FP control”, “Low FP control”, and “Reference”, prepare the Diluent Solution of 10% DMSO (aqueous) without any test inhibitor compound. The final concentration of DMSO will be 1% in all samples. If the compound was dissolved in another diluent, use that diluent to prepare the control diluent solution.

4) Prepare the PARP1 enzyme:
   Thaw **PARP1** enzyme on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Calculate the amount of PARP1 required for the assay and 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 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.

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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 1	10 µl	10 µl	10 µl	10 µl
Test Inhibitor	-	–	2.5 µl	–
Diluent solution (no inhibitor)	2.5 µl	2.5 µl	–	2.5 µl
1x PARPtrap™ assay buffer	-	–	–	10 µl
PARP1 (0.5 ng/µl) as prepared in step 3	10 µl	10 µl	10 µl	–
Total	22.5 µl	22.5 µl	22.5 µl	22.5 µl

6) To the wells designated as "Blank-PARP1", add 5 µl of **5x PARPtrap™ assay buffer** + 15 µl of distilled water + 2.5 µl of diluent (for example 10% DMSO).

Note that the "Blank-PARP1" contains buffer components of the reaction but does not contain the fluorescent probe or PARP1.

	Blank-PARP1
5x PARPtrap™ assay buffer	5 µl
Water	15 µl
Diluent solution (no inhibitor)	2.5 µl
Total	22.5 µl

7) If PARP2-containing reactions are not performed go directly to step 15.

For reactions containing PARP2 enzyme

8) Calculate the amount of **5x PARPtrap™ assay buffer 2** required for the assay and add fresh DTT to a 10 mM concentration (note that DTT concentration in the master mix and in the 1x assay buffer 2 will be 2 mM).

9) Dilute **100 nM Fluorescent labeled nicked DNA** 8-fold to 12.5 nM before use. Prepare the Master Mix 2 (10 µl per well): N wells x (3 µl of **5x PARPtrap™ assay buffer 2** (with DTT) + 0.5 µl of **12.5 nM Fluorescent labeled nicked DNA** + 6.5 µl of distilled water). For example, for 40 wells prepare: 120 µl of 5x PARPtrap™ assay buffer 2 with DTT+ 20 µl of 12.5 nM Fluorescent labeled nicked DNA + 260 µl of distilled water.

10) Prepare **1x PARPtrap™ assay buffer 2** by diluting one part of **5x PARPtrap™ assay buffer 2** (with DTT) with four parts of distilled water. Dilute only enough required for the assay.

11) Prepare the inhibitor solution (if different than in Step 3).
If the inhibitor compound is dissolved in DMSO, make a 10-fold dilution in water so the final concentration of DMSO will not exceed 1% in all samples.
Prepare the control diluent solution: for the wells labeled "High FP control", "Low FP control", and "Reference", prepare the diluent Solution of 10% DMSO (aqueous) without any test inhibitor compound. The

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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.

12) Prepare the PARP2 enzyme:
Thaw **PARP2** enzyme on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Calculate the amount of PARP2 required for the assay and dilute the enzyme to ~3.75 ng/µl with **1x PARPtrap™ assay buffer 2** (with DTT).
Note: **PARP2** 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 PARP2 required for the assay, dilute only the amount sufficient for the assay and aliquot the remaining undiluted **PARP2** enzyme. Store single use aliquots at -80°C. Do not re-use a thawed aliquot or diluted enzyme.

13) 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 2	10 µl	10 µl	10 µl	10 µl
Test Inhibitor	-	–	2.5 µl	–
Diluent solution (no inhibitor)	2.5 µl	2.5 µl	–	2.5 µl
1x PARPtrap™ assay buffer 2	-	–	–	10 µl
PARP2 (3.75 ng/µl) as prepared in step 3	10 µl	10 µl	10 µl	–
Total	22.5 µl	22.5 µl	22.5 µl	22.5 µl

14) To the wells designated as "Blank-PARP2", add 5 µl of **5x PARPtrap™ assay buffer 2** (with DTT) + 15 µl of distilled water + 2.5 µl of diluent (for example DMSO 10%)

Note that the "Blank-PARP2" contains buffer components of the reaction but does not contain the fluorescent probe or PARP2.

	Blank-PARP2
5x PARPtrap™ assay buffer 2	5 µl
Water	15 µl
Diluent solution (no inhibitor)	2.5 µl
Total	22.5 µl

Notes:

- Reference: a negative control lacking PARP1 or PARP2, in which all the fluorescent DNA is present in free form and FP is lowest (*this is different from the Blanks 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 PARP1 or PARP2 that is bound to fluorescent DNA cannot not undergo ribosylation and remains associated to the DNA, resulting in the maximum FP allowed by the kit.

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- Low FP control: a condition in which all PARP1 or PARP2 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 PARP1/2 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 (or other diluent) as used to prepare the test inhibitor, but without inhibitor

Incubation Steps

For all reactions:

15) After assembling kit components according to the tables, incubate at room temperature for 30-60 minutes.

16) To the wells indicated as “High FP control”, add 2.5 µl distilled water**.**

17) Initiate PARP1/2 enzymatic reaction by adding 2.5 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 25 µl.
    Incubate the plate for 60 min at room temperature.

18) 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 values are subtracted from all other values.

Example Results

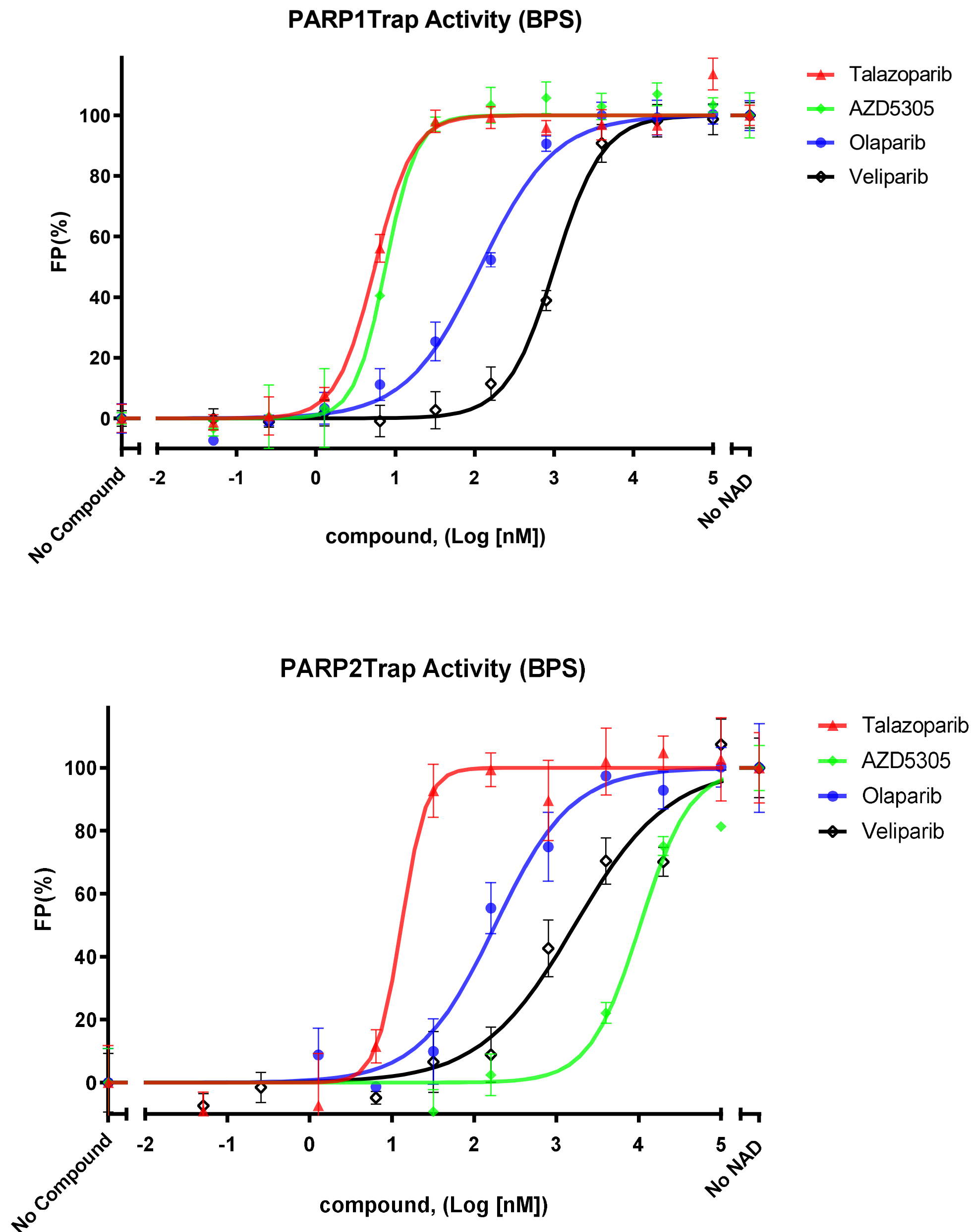

Figure 1: PARP1/2 trapping on DNA measured in the presence of increasing concentrations of Talazoparib (Selleckchem), Olaparib (LC Laboratories), Veliparib (Selleckchem) and AZD5305 (MedChemExpress) using the PARPtrap™ Assay Kits, BPS Bioscience, #80584 & #78296. "No compound" corresponds to the "Low 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 support@bpsbioscience.com

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CALCULATING RESULTS:

Definition of Fluorescence Polarization:

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

where $I_{//}$ = 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".

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

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 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):** 30-31

Related Products

Products	*Catalog #*	*Size*
PARPtrap™ Assay Kit for PARP1	80584	96 & 384 rxns
PARPtrap™ Assay Kit for PARP2	78296	96 & 384 rxns
Set of PARP inhibitors	78318	8 x 50 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