Description

The PARPtrap™ Combo Assay Kit for PARP1 and PARP2 is a homogeneous fluorescence polarization (FP) assay designed to measure PARP1 (poly (ADP-ribose) polymerase 1) and PARP2 (poly (ADP-ribose) polymerase 2) DNA complex formation in a high throughput screening (HTS) format, allowing to measure PARP1/2 trapping. The PARPtrap™ Combo Assay Kit for PARP1 and PARP2 comes in a convenient 384-well format, with enough purified recombinant PARP1 enzyme, PARP2 (amino acids 2-583), a fluorescent-labeled oligonucleotide duplex, NAD^+ and PARPtrap™ assay buffer for 400 enzyme reactions.

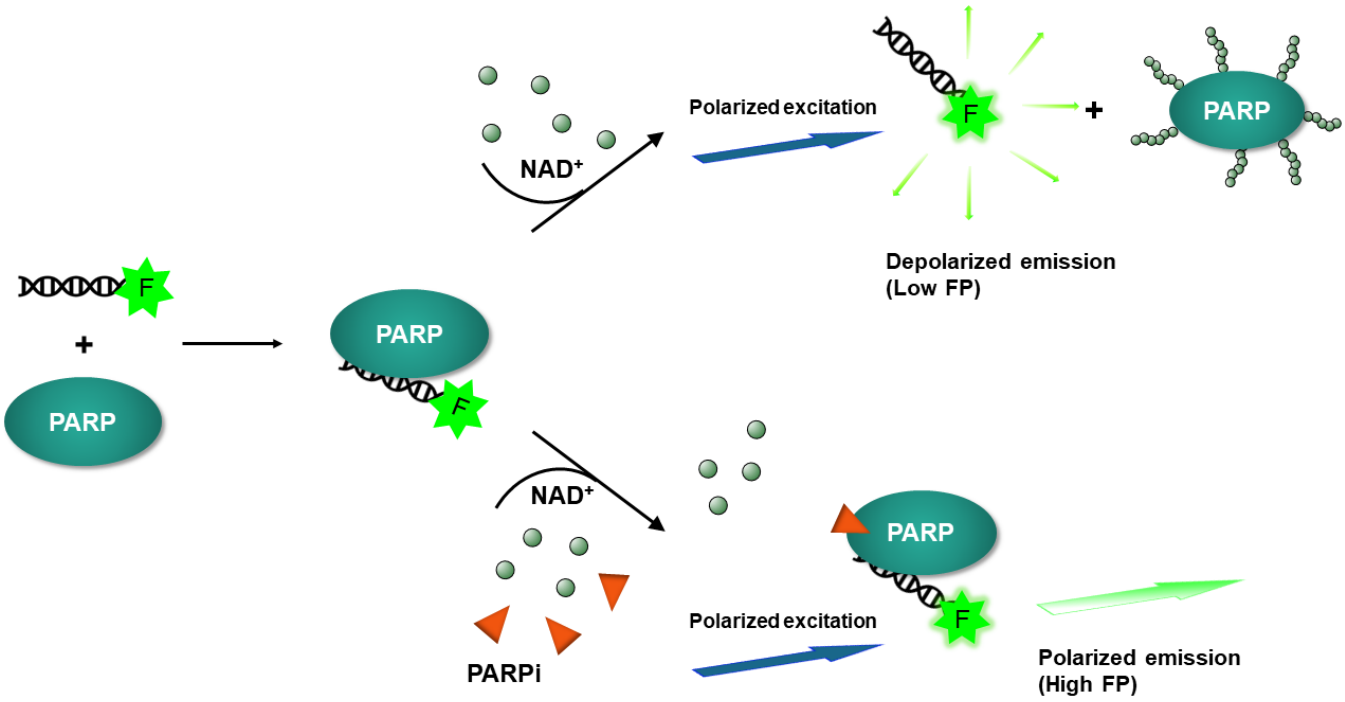


Figure 1. Schematic representation of the mechanism behind the PARPtrap™ Combo Assay Kit for PARP1 and PARP2.

The Fluorescent Labeled Oligonucleotide Duplexes are small fluorescent probes that can rotate freely in solution, and thus have a low FP. In the absence of ribosylation, PARP1/2 binds to its fluorescent probe, forming a large complex. In the presence of NAD^+ PARP1/2 undergoes auto-ribosylation and dissociates from the fluorescent oligonucleotide duplex, which can then rotate freely again (low FP). In the presence of a PARP1/2 inhibitor, PARP1/2 can be trapped to the fluorescent oligonucleotide duplex and result in a high FP. The increase in FP signal is directly proportional to PARP1/2 trapping.

This assay requires a fluorescent microplate reader ***capable of measuring fluorescence polarization (FP)*** to read the FP signal. For more information on FP technology, visit our Tech Note: FP, assay principles and applications.

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Background

PARP1, also known as poly-(ADP-ribose) polymerase 1 or NAD^+ ADP-ribosyltransferase 1, is part of the PARP family, and it is the most abundant member. ADP ribosylation, which is the addition of an ADP-ribose to a protein, is a reversible post-translational modification of proteins mostly involved in the DNA Damage Response (DDR) pathway. Poly-ADP-ribosylation (termed PARylation) is the addition of linear or branched chains of ADP-ribose. PARP1 participates in DNA repair by non-homologous end joining (NHEJ), homologous recombination (HR), microhomology-mediated end-joining (MMEJ) and nucleotide excision repair. Dysfunction of DDR pathways can lead to oncogenesis. Overexpression of PARP1 has been found in breast and colon cancer, neuroblastoma, and others. This overexpression can lead to increasing MMEJ, an error-prone DNA repair mechanism, and genome instability leading to cancer. In addition to being involved in DDR, PARP1 is also linked to inflammation and type I diabetes. PARP2 participates in DNA repair (only 10% of the PARP activity is due to PARP2), but also in oxidative stress and mitochondrial fragmentation. Dysfunction of the DDR and oxidative stress pathways can lead to oncogenesis. Genetic ablation of PARP2 has indicated roles of PARP2 in adipogenesis, spermatogenesis and thymocyte survival. It is also a co-factor of nuclear receptors like ER (estrogen receptor) and PPAR (peroxisome proliferator-activated receptors). PARP2 is overexpressed in prostate cancer (PCa) and may contribute to the disease through the FOXA1 (forkhead box protein A1)/AR pathway. PARP1 and PARP2 are known to bind damaged DNA through its DNA-binding domains. Binding to DNA activates PARP1/2 and in the presence of NAD^+ PARP1/2 ribosylates itself (auto-ribosylation) and dissociates from the DNA due to the accumulated negative charge of the ribosyl polymer. In the presence of some inhibitors, however, PARP remains bound to DNA, a phenomenon termed trapping. Trapped PARP-DNA complexes have been shown to be highly cytotoxic to cancer cells, making such inhibitors desirable in cancer therapy. Further understanding of the molecular pathways involving PARP1/2, and their contribution to disease, will continue to pave the way for new therapies for PARP1/2-linked diseases.

Applications

Screen small molecules that enhance PARP1/2 trapping onto DNA for drug discovery and high throughput screening (HTS) applications, and IC_{50} determination.

Supplied Materials

	Catalog #	Name	Amount	Storage
PARP1 Specific Reagents	83582-KC1	PARP1, GST-Tag*	1 µg	-80°C
	78273-KC100	25 nM Fluorescent Labeled Oligonucleotide Duplex	2 x 100 µl	-80°C
	82674-KC1	5x PARPtrap™ Assay Buffer	2 x 1 ml	-80°C
PARP2 Specific Reagents	83540-KC8	PARP2, GST-Tag*	8 µg	-80°C
	78297-KC12.5	100 nM Fluorescent Labeled Nicked Oligonucleotide Duplex	2 x 12.5 µl	-80°C
	82675-KC1	5x PARPtrap™ Assay Buffer 2	2 x 1 ml	-80°C
	82735-KC200	0.5 M DTT	200 µl	-20°C
	82676-KC500	10x NAD^+	2 x 500 µl	-80°C
	79961	Black 384-well plate	1	Room Temp

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** The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein. The protein formulation has been optimized for performance in this assay.*

Materials Required but Not Supplied

- Nuclease-Free Distilled Water
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- Fluorescent microplate reader capable of measuring fluorescence polarization (λex=470 (5 nm bandwidth) and detection at λem=518 (10 nm bandwidth)

Storage Conditions



This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

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 final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Reference Control” (no PARP1/2, all fluorescent oligonucleotide duplex is in the free form and has low FP, minimum FP value), “Low FP Control” (all PARP1/2 dissociates from the fluorescent oligonucleotide duplex), “High FP Control” (no NAD^+, all PARP1/2 remains bound to the fluorescent oligonucleotide duplex, maximum FP value) and “Test Inhibitor” conditions.
- If measuring PARP1 and PARP2 trapping in parallel prepare two sets of controls, one for each PARP, following the protocols described below.
- Please note that PARP1 and PARP2 trapping use different buffers and Fluorescent Oligonucleotide Duplex.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Talazoparib (Set of PARP Inhibitors (8 x 50 µl), #78318) as internal controls. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Protocol 1: PARP1 Trapping

1. Prepare a **Master Mix 1** (10 µl/well, except "Blank" wells): N wells x (3 µl of 5x PARPtrap™ Assay Buffer + 1 µl of 25 nM Fluorescent Labeled Oligonucleotide Duplex + 6 µl of distilled water).

2. Prepare a **Deficient Master Mix 1** (10 µl/"Blank" well): N wells x (3 µl of 5x PARPtrap™ Assay Buffer + 7 µl of distilled water).

3. Prepare **1x PARPtrap™ Assay Buffer** by diluting 5-fold the 5x PARPtrap™ Assay Buffer with distilled water.

4. Prepare the **Test Inhibitor** (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   4.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in distilled water, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use distilled water (Diluent Solution).

   OR

   4.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in distilled water to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in distilled water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

5. Thaw **PARP1** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

6. Dilute **PARP1** to 0.5 ng/µl with 1x PARPtrap™ Assay Buffer (10 µl/well).

7. Add 10 µl of **Master Mix 1** to each well, except "Blank" wells.

8. Add 10 µl of **Deficient Master Mix 1** to the "Blank" wells.

9. Add 2.5 µl of diluted **inhibitor** to the "Test Inhibitor" wells.

10. Add 2.5 µl of **Diluent Solution** to the remaining wells.

11. Add 10 µl of **diluted PARP1** to the "High FP Control", "Low FP Control" and "Test Inhibitor" wells.

12. Add 10 µl of **1x PARPtrap™ Assay Buffer** to the "Reference Control" and "Blank" wells.

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13. Incubate at Room Temperature (RT) for 30 minutes.

    Note: If needed, the formation of PARP1/DNA complexes can be verified by measuring fluorescence polarization at this step. High FP values are expected for "High FP Control", "Low FP Control" and "Test Inhibitor" wells.

14. Add 2.5 µl of **distilled water** to the "High FP Control" wells.

15. Initiate the reactions by adding 2.5 µl of **10x NAD$^+$** to the "Low FP Control", "Test Inhibitor", "Reference Control" and "Blank" wells**.**

	High FP Control	Low FP Control	Test Inhibitor	Reference Control	Blank
Master Mix 1	10 µl	10 µl	10 µl	10µl	-
Deficient Master Mix 1	-	-	-	-	10 µl
Test Inhibitor	-	-	2.5 µl	-	-
Diluent Solution	2.5 µl	2.5 µl	-	2.5 µl	2.5 µl
1x PARPtrap™ Assay Buffer	-	-	-	10 µl	10 µl
Diluted PARP1 (0.5 ng/µl)	10 µl	10 µl	10 µl	-	-
10x NAD$^+$	-	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Distilled Water	2.5 µl	-	-	-	-
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**	**25 µl**

16. Incubate the plate for 60 minutes at RT.

17. Read FP in a fluorescence plate reader capable of measuring fluorescence polarization (λex = 470 (5 nm bandwidth); λem = 518 (10 nm bandwidth) **and set to FP**.

18. Subtract the "Blank" value from all other values.

Protocol 2: PARP2 Trapping

1. Dilute 0.5 M DTT 50-fold with 5x PARPtrap™ Assay Buffer 2. This makes a **5x PARPtrap™ Assay Buffer with 10 mM DTT**.

2. Dilute **100 nM Fluorescent Labeled Nicked Oligonucleotide Duplex** 4-fold with distilled water (0.5 µl/well).

3. Prepare a **Master Mix 2** (10 µl/well, except "Blank" wells): N wells x (3 µl of 5x PARPtrap™ Assay Buffer 2 with DTT + 0.5 µl of diluted Fluorescent Labeled Nicked Oligonucleotide Duplex + 6.5 µl of distilled water).

4. Prepare a **Deficient Master Mix 2** (10 µl/ "Blank" well): N wells x (3 µl of 5x PARPtrap™ Assay Buffer with DTT + 7 µl of distilled water).

5. Prepare **1x PARPtrap™ Assay Buffer 2** by diluting 5-fold the 5x PARPtrap™ Assay Buffer 2 with DTT with distilled water.

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6. Prepare the **Test Inhibitor** (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   6.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in distilled water, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use distilled water (Diluent Solution).

   OR

   6.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in distilled water to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in distilled water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

7. Thaw **PARP2** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

8. Dilute **PARP2** to 3.75 ng/µl with 1x PARPtrap™ Assay Buffer 2 (10 µl/well).

9. Add 10 µl of **Master Mix 2** to each well, except "Blank" wells.

10. Add 10 µl of **Deficient Master Mix 2** to the "Blank" wells.

11. Add 2.5 µl of diluted **inhibitor** to the "Test Inhibitor" wells.

12. Add 2.5 µl of **Diluent Solution** to the remaining wells.

13. Add 10 µl of **diluted PARP2** to the "High FP Control", "Low FP Control" and "Test Inhibitor" wells.

14. Add 10 µl of **1x PARPtrap™ Assay Buffer 2** to the "Reference" and "Blank" wells.

15. Incubate at Room Temperature (RT) for 30 minutes.

    Note: If needed, the formation of PARP2/DNA complexes can be verified by measuring fluorescence polarization at this step. High FP values are expected for "High FP Control", "Low FP Control" and "Test Inhibitor" wells.

16. Add 2.5 µl of distilled **water** to the "High FP Control" wells.

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17. Initiate the reactions by adding 2.5 µl of **10x NAD$^+$** to the "Low FP Control", "Test Inhibitor", "Reference Control" and "Blank" wells**.**

	High FP Control	Low FP Control	Test Inhibitor	Reference	Blank
Master Mix 2	10 µl	10 µl	10 µl	10 µl	-
Deficient Master Mix 2	-	-	-	-	10 µl
Test Inhibitor	-	-	2.5 µl	-	-
Diluent Solution	2.5 µl	2.5 µl	-	2.5 µl	2.5 µl
1x PARPtrap™ Assay Buffer 2	-	–	-	10 µl	10 µl
Diluted PARP2 (3.75 ng/µl)	10 µl	10 µl	10 µl	-	-
10x NAD$^+$	-	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Distilled Water	2.5 µl	-	-	-	-
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**	**25 µl**

18. Incubate the plate for 60 minutes at RT.

19. Read FP in a fluorescence plate reader capable of measuring fluorescence polarization (λex = 470 (5 nm bandwidth); λem = 518 (10 nm bandwidth) **and set to FP**.

20. Subtract the "Blank" value from all other values.

Calculating Results

Fluorescence polarization is a measure of the amount of molecular rotation that takes place in the time between excitation and emission of the fluorescence probe. It can be determined from the measurements of perpendicular ($I_\perp$) and parallel (I_{II}) fluorescence intensity values emitted by the probe relative to the direction of the polarized excitation light (Figure 2).

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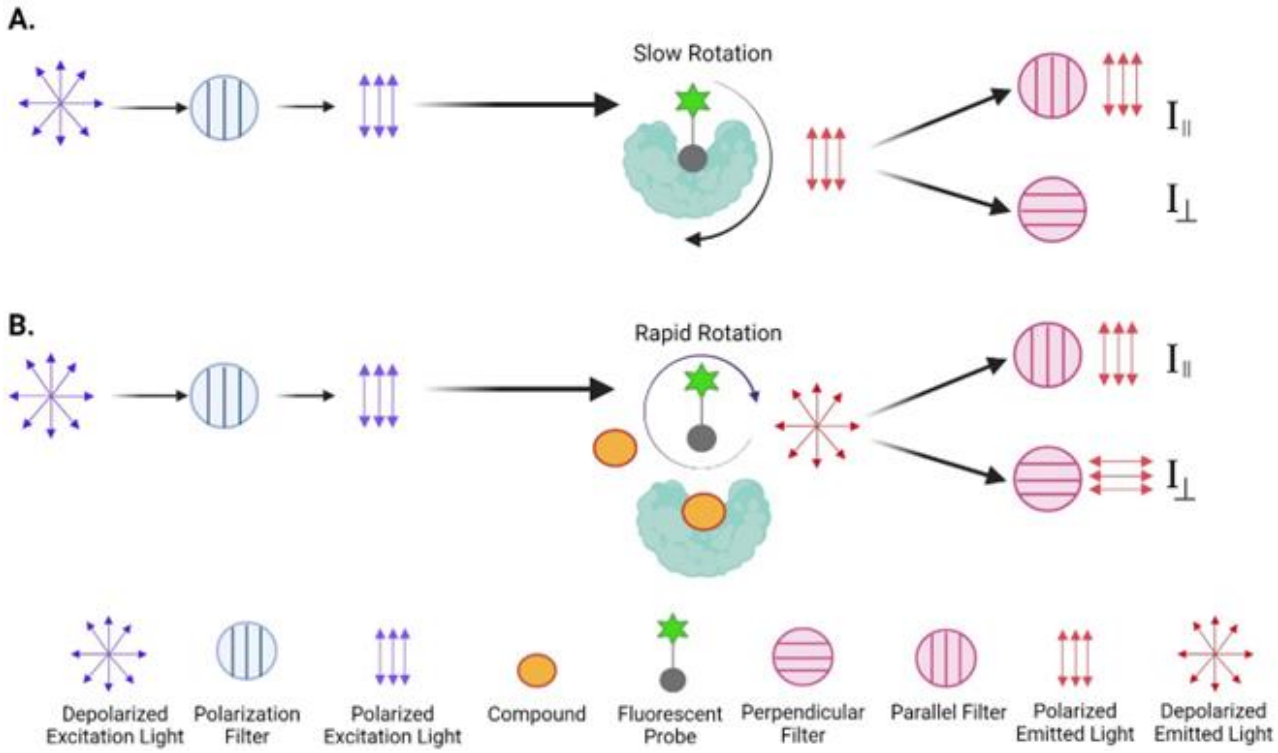


Figure 2: Fluorescence polarization principle.
A. When the fluorescently labeled probe binds to a larger protein it creates a complex of a big molecular weight that has a slow rotation ability. In this state the probe has a reduced rotational diffusion so when it is excited by polarized light, it still emits highly polarized light with a degree of polarization that is inversely proportional to the rate of molecular rotation.
B. In the presence of a compound that has affinity for the protein, the fluorescent probe remains in solution and can rotate rapidly. Unbound probe has a high rotational diffusion so when it is excited by the polarized light it emits light in orientations that can be detected by both the perpendicular and parallel filters.

Polarization is defined as the difference between the emission intensities of parallel fluorescence ($I_{||}$) and perpendicular fluorescence ($I_{\perp}$), divided by the total fluorescence emission intensity. The polarization value (P) being a ratio of light intensities, is a dimensionless number, often expressed in milli P units where 1 P unit = 1000 mP units. To calculate P one has to take into consideration that light is not transmitted equally well through both parallel and perpendicular channels and therefore a correction must be made. This correction factor is called the "G Factor" (G) and it is specific to the instrument used. mP can thus be calculated using the following formula:

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

Modern instruments usually have the G factor pre-calculated and can automatically calculate fluorescence polarization for your experiments. If you need to determine, set up or calculate the G factor please refer to your instrument manual (the instrument manual should contain information about how to establish the G-factor) or check our FAQ section (FAQs (bpsbioscience.com).

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For accurate calculations it is necessary to provide the correct plate schematic when setting up your instrument, with defined positions for the "Blank" and "Reference" (also known as Substrate Control) wells, and to ensure that the emission intensities from the "Blank" wells are subtracted from all other wells prior to further data analysis.

We encourage you to analyze raw data and if appropriate to exclude those "Blank" or "Reference" wells that show aberrant readouts prior to mP determination.

The % of Activity can be calculated as follows:

$$\%\ of\ Activity = \frac{(mP\ value\ from\ Test\ Inhibitor - mP\ value\ from\ Reference\ Control)}{(mP\ value\ from\ Positive\ Control - mP\ value\ from\ Reference\ Control)} x\ 100$$

Example Results

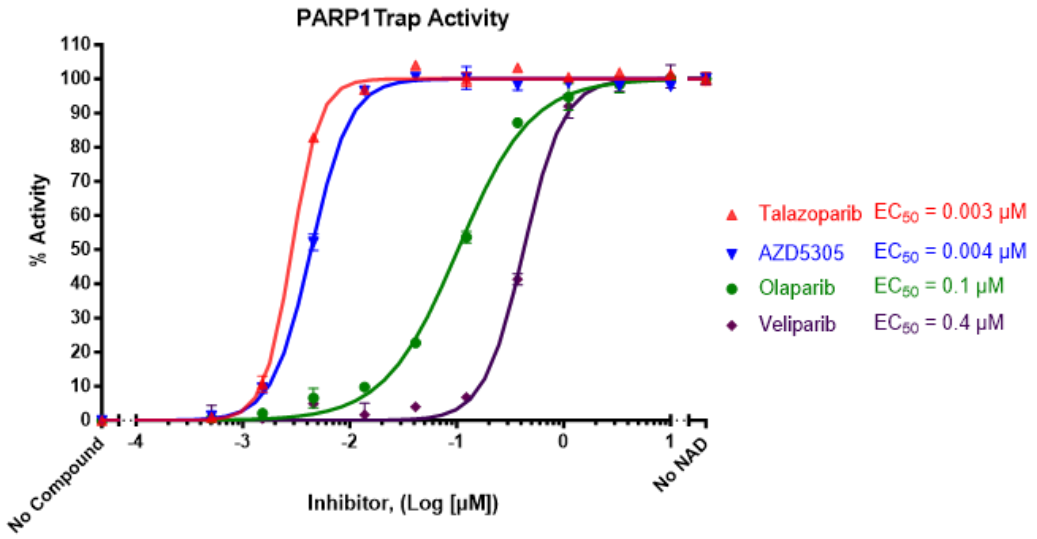


Figure 3: PARP1 trapping to DNA by Talazoparib, AZD5305, Olaparib and Veliparib.
PARP1 trapping onto DNA was measured in the presence of increasing concentrations of Talazoparib, Olaparib, Veliparib and AZD5305 (#78318). "No compound" corresponds to the "Low FP Control" and "no NAD" corresponds to the "High FP Control" conditions.

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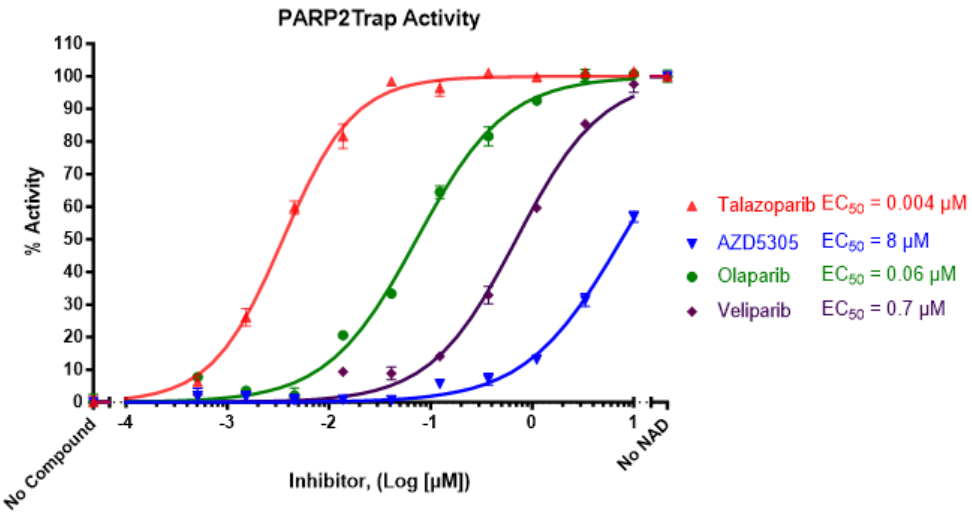


Figure 4: PARP2 trapping to DNA by Talazoparib, AZD5305, Olaparib and Veliparib.
PARP2 trapping onto DNA was measured in the presence of increasing concentrations of Talazoparib, Olaparib, Veliparib and AZD5305 (#78318). "No compound" corresponds to the "Low FP Control" and "no NAD" corresponds to the "High FP Control" conditions.

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
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Murai J., *et. al*., 2012*Cancer Research* 72**:** 5588-5599
Zandarashvili L., *et al*., 2020 *Science* 368(6486): 6367
Marques M., *et al.*, 2019 *Oncogene* 38 (12): 2177-2191.
Gui B., *et al.*, 2019 *Proc Natl Acad Sci USA* 116 (29): 14573-14582.

Related Products

Products	*Catalog #*	*Size*
PARPtrap™ Assay Kit for PARP1	80584	96 reactions/384 reactions
PARPtrap™ Assay Kit for PARP2	78296	96 reactions/384 reactions
PARP1 Colorimetric Assay Kit	80580	96 reactions
PARP1 Homogeneous Assay Kit	78438	384 reactions
PARP2 Chemiluminescent Assay Kit	80552	96 reactions
PARP3 Chemiluminescent Assay Kit	80553	96 reactions/384 reactions

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