

Description

The PARPtrap™ Assay Kit for PARP2 is a fluorescence polarization (FP), homogeneous, 96-well assay designed for screening and profiling small molecules that trap PARP2 onto DNA and complex formation in a high throughput screening (HTS) format. The PARPtrap™ Assay Kit for PARP2 comes in a convenient 96-well format, with purified recombinant PARP2 enzyme (amino acids 2-583), a fluorescently-labeled oligonucleotide duplex, NAD⁺ and PARPtrap™ assay buffer for 100 enzyme reactions.

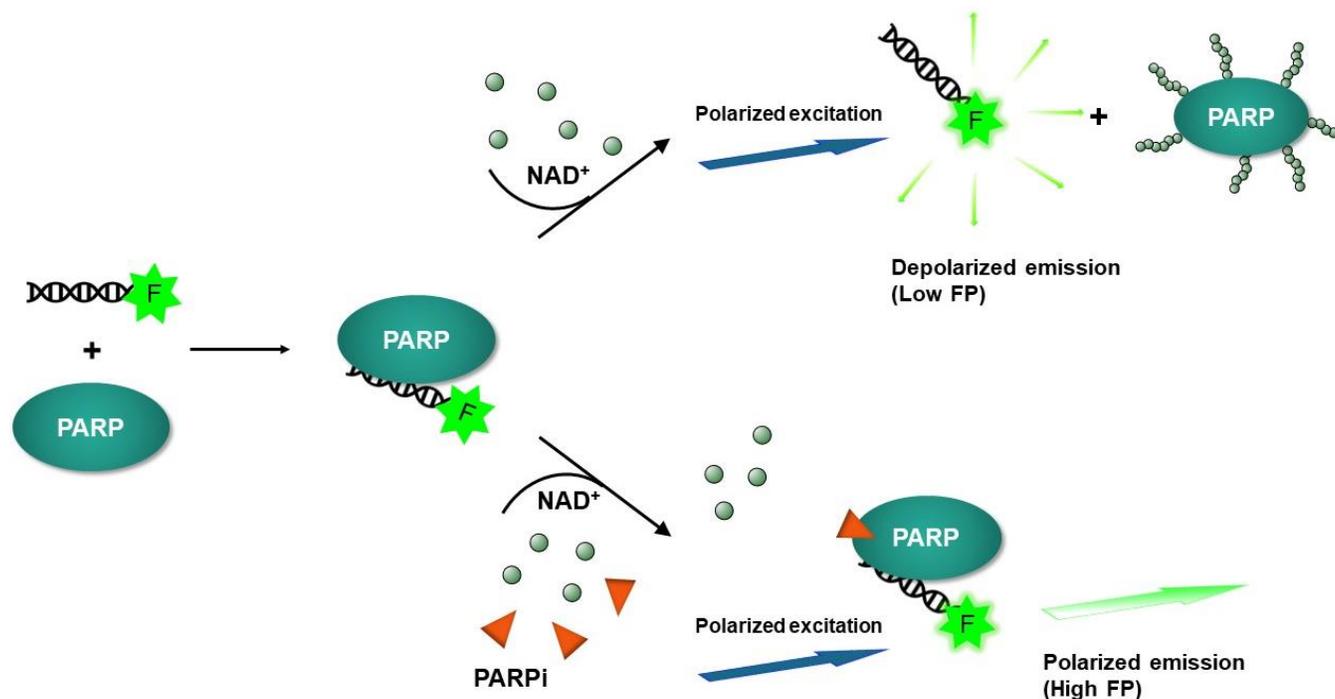


Figure 1. Diagram illustration the mode of action of PARPtrap™ Assay Kit for PARP2.

The fluorescent probe is a small molecule that can rotate freely in solution, resulting in low FP. In the absence of ribosylation, PARP2 binds to the fluorescent probe, forming a large complex and resulting in the emission of highly polarized light. After the auto-ribosylation step, PARP2 dissociates from the fluorescent oligonucleotide duplex, which can then rotate freely again (low FP). In the presence of a PARP2 inhibitor, PARP2 is trapped to the fluorescent oligonucleotide duplex, having a high FP. The increase in FP signal is directly proportional to PARP trapping.

This assay requires a fluorescent microplate reader capable of measuring fluorescence polarization (FP) and equipped with the required parts to read the FP signal. For more information FP technology, visit our Tech Note: [FP, assay principles and applications](#).

Background

PARP2, also known as poly-(ADP-ribose) polymerase 2 or NAD⁺ ADP-ribosyltransferase 2, is part of the PARP family. 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. 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 DDR and oxidative stress pathways can lead to oncogenesis. Genetic ablation of PARP2 has shown specific roles of PARP2 in adipogenesis, spermatogenesis and thymocyte survival. It also functions as a co-factor to 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. PARP2 is known to bind damaged DNA through its DNA-binding domains. Binding to DNA activates PARP2 and in the presence of NAD⁺ PARP2 ribosylates itself (auto-ribosylation), what in consequence leads to PARP2 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 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. Further understanding of the molecular pathways involving PARP2, and this contribution to disease, will continue to pave the way for new therapies for PARP2-linked diseases.

Applications

Screen small molecules that enhance PARP2/DNA trapping for drug discovery and high throughput screening (HTS) applications, and IC₅₀ determination.

Supplied Materials

Catalog #	Name	Amount	Storage
83540-KC8	PARP2, GST-Tag*	8 µg	-80°C
78297-KC12.5	100 nM Fluorescent Labeled Nicked Oligonucleotide Duplex	12.5 µl	-80°C
82675-KC1	5x PARPtrap™ Assay Buffer 2	2 x 1 ml	-80°C
82676-KC500	10x NAD ⁺	500 µl	-80°C
82735-KC200	0.5 M DTT	200 µl	-20°C
79685	Black 96-well plate	1 plate	Room Temp

* 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

- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- Fluorescent microplate reader capable of measuring fluorescence polarization ($\lambda_{\text{exc}}=470$ (5 nm bandwidth) and detection at $\lambda_{\text{em}}=518$ (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 PARP2), “Low FP Control” (all PARP2 dissociates from the fluorescent oligonucleotide duplex), “High FP Control” (no NAD⁺, all PARP2 remains bound to the fluorescent oligonucleotide duplex) and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to [Protein FAQs \(bpsbioscience.com\)](https://www.bpsbioscience.com/protein-faqs).
- We recommend using Talazoparib (Set of PARP Inhibitors (8 x 50 µl), #78318) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC₅₀ value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://www.bpsbioscience.com/serial-dilution-protocol).

1. Dilute **0.5 M DTT** 50-fold with 5x PARPtrap™ Assay Buffer 2. This makes **5x PARPtrap™ Assay Buffer 2 with DTT**.
2. Dilute **100 nM Fluorescent Labeled Nicked Oligonucleotide Duplex** 8-fold with distilled water (1 µl/well).

Note: Prepare only the amount needed. The remaining 100 nM Fluorescent Labeled Nicked Oligonucleotide Duplex can be stored in single use aliquots (minimum volume of 5 µl/aliquot) at -80°C.

3. Prepare a **Master Mix** (20 µl/well, except “Blank” wells): N wells x (6 µl of 5x PARPtrap™ Assay Buffer with DTT + 1 µl of diluted Fluorescent Labeled Nicked Oligonucleotide Duplex + 13 µl of distilled water).
4. Prepare a **Deficient Master Mix** (20 µl for each “Blank” well): N wells x (6 µl of 5x PARPtrap™ Assay Buffer + 14 µl of distilled water).
5. Prepare **1x PARPtrap™ Assay Buffer** by diluting 5x PARPtrap™ Assay Buffer with DTT 5-fold with distilled water.

6. Prepare the **Test Inhibitor** (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 50 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 100-fold in distilled water to prepare the highest concentration of the 100-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 (20 µl/well). For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).
9. Add 20 µl of **Master Mix** to each well, except “Blank” wells.
10. Add 20 µl of **Deficient Master Mix** to the “Blank” wells.
11. Add 5 µl of **diluted inhibitor** to the “Test Inhibitor” wells.
12. Add 5 µl of **Diluent Solution** to the remaining wells.
13. Add 20 µl of **diluted PARP2** to the “High FP Control”, “Low FP Control” and “Test Inhibitor” wells.
14. Add 20 µl of **1x PARPtrap™ Assay Buffer** to the “Reference” and “Blank” wells.
15. Incubate at Room Temperature (RT) for 30-60 minutes.
16. Add 5 µl of **distilled water** to the “High FP Control” wells.
17. Initiate the reactions by adding 5 µl of **10x NAD⁺** to all other wells, except the “High FP Control”.

	High FP Control	Low FP Control	Test Inhibitor	Reference	Blank
Master Mix	20 μ l	20 μ l	20 μ l	20 μ l	-
Deficient Master Mix	-	-	-	-	20 μ l
Test Inhibitor	-	-	5 μ l	-	-
Diluent Solution	5 μ l	5 μ l	-	5 μ l	5 μ l
1x PARPtrap™ Assay Buffer	-	-	-	20 μ l	20 μ l
Diluted PARP2 (3.75 ng/ μ l)	20 μ l	20 μ l	20 μ l	-	-
Room Temperature, 30-60 minutes					
10x NAD ⁺	-	5 μ l	5 μ l	5 μ l	5 μ l
Distilled Water	5 μ l	-	-	-	-
Total	50 μl				

18. Incubate the plate for 60 minutes at RT.

19. Read FP in a fluorescence plate reader capable of measuring fluorescence polarization (λ_{ex} = 470 (5 nm bandwidth); λ_{em} = 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_{\parallel}) fluorescence intensity values emitted by the probe relative to the direction of the polarized excitation light (Figure 2).

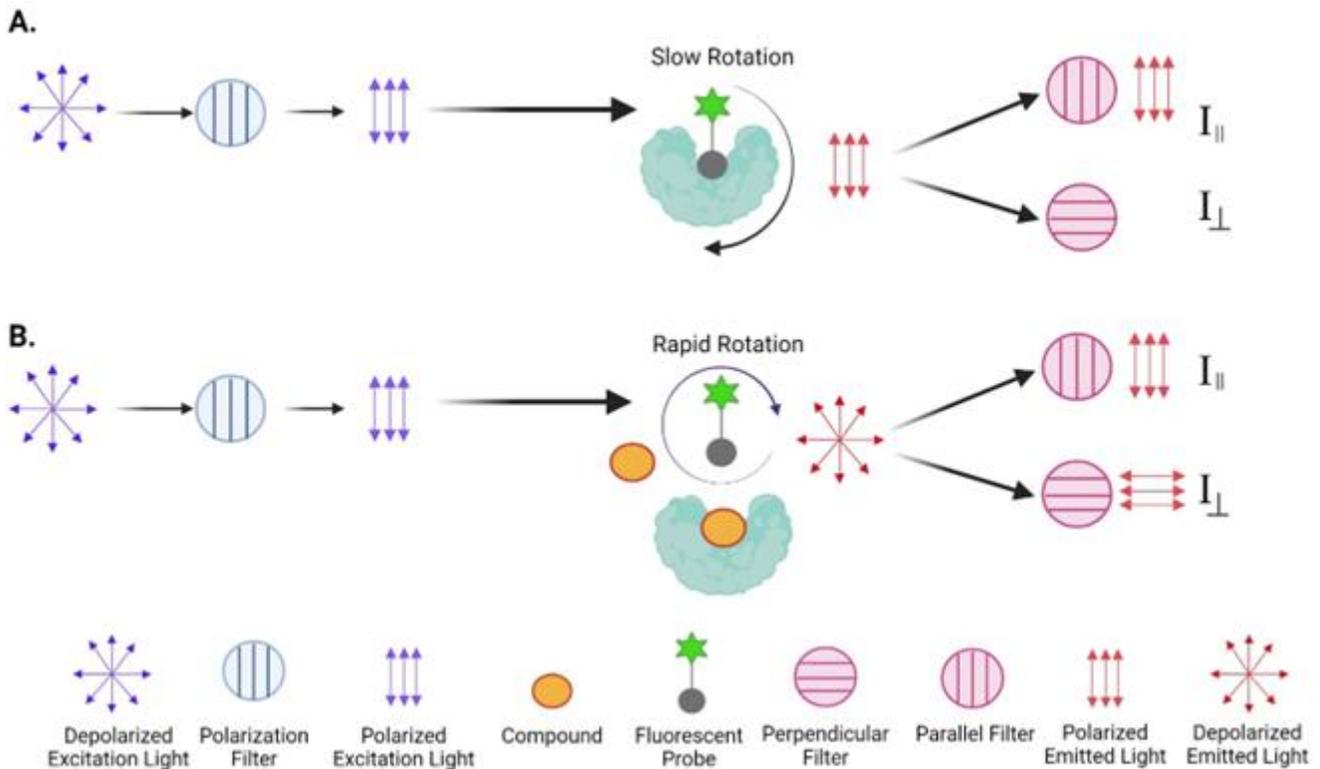


Figure 2: Fluorescence polarization principle.

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_⊥), 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:

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

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 \(bpsbioscience.com\)](https://www.bpsbioscience.com/FAQs)).

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:

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

Example Results

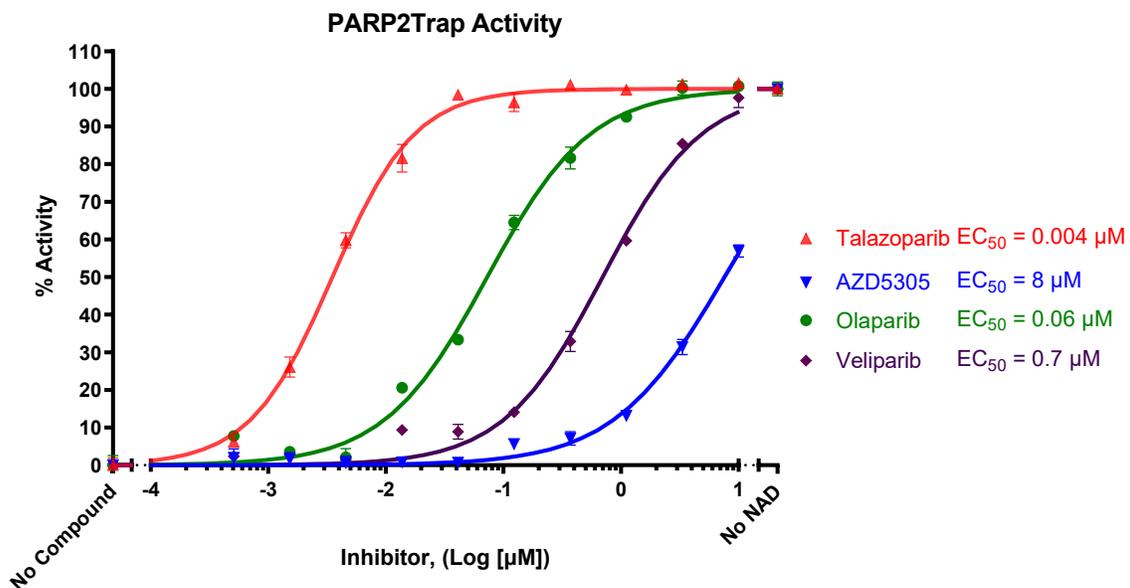


Figure 1: PARP2 trapping by Talazoparib, AZD5305, Olaparib and Veliparib.

The PARP2 trapping onto DNA was measured in the presence of increasing concentrations of Talazoparib, Olaparib, Veliparib and AZD5305 (all available as part of [Set of PARP Inhibitors \(8 x 50 µl\)](#), BPS Bioscience #78318). “No compound” corresponds to the “Low FP control” and “no NAD” corresponds to the “High FP control”.

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

Murai J., *et al.*, 2014 *Molecular Cancer Therapeutics* 13: 433-443

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.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
PARPtrap™ Combo Assay Kit for PARP1 and PARP2	78317	384 reactions
PARPtrap™ Assay Kit for PARP1	80584	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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