Description

The PARP1 Olaparib Competitive Inhibitor Assay Kit is a competitive FP (fluorescent polarization) assay designed to measure the formation of a complex between PARP1 (poly(ADP-ribose) polymerase 1) and a fluorescent probe that contains the PARP1 inhibitor Olaparib. When the probe is bound to PARP1, FP is high. In the presence of a test compound able to bind to the same site in PARP1 as Olaparib, the Olaparib-containing fluorescent probe is displaced from PARP1 and remains in solution, resulting in low FP. The PARP1 Olaparib Competitive Inhibitor Assay Kit comes in a convenient 96-well format, with purified PARP1 enzyme, Olaparib-containing fluorescent-labeled probe, and assay buffer for 100 enzyme reactions.

Note: This kit is not appropriate for inhibitors expected to bind to PARP1 at different sites from Olaparib.

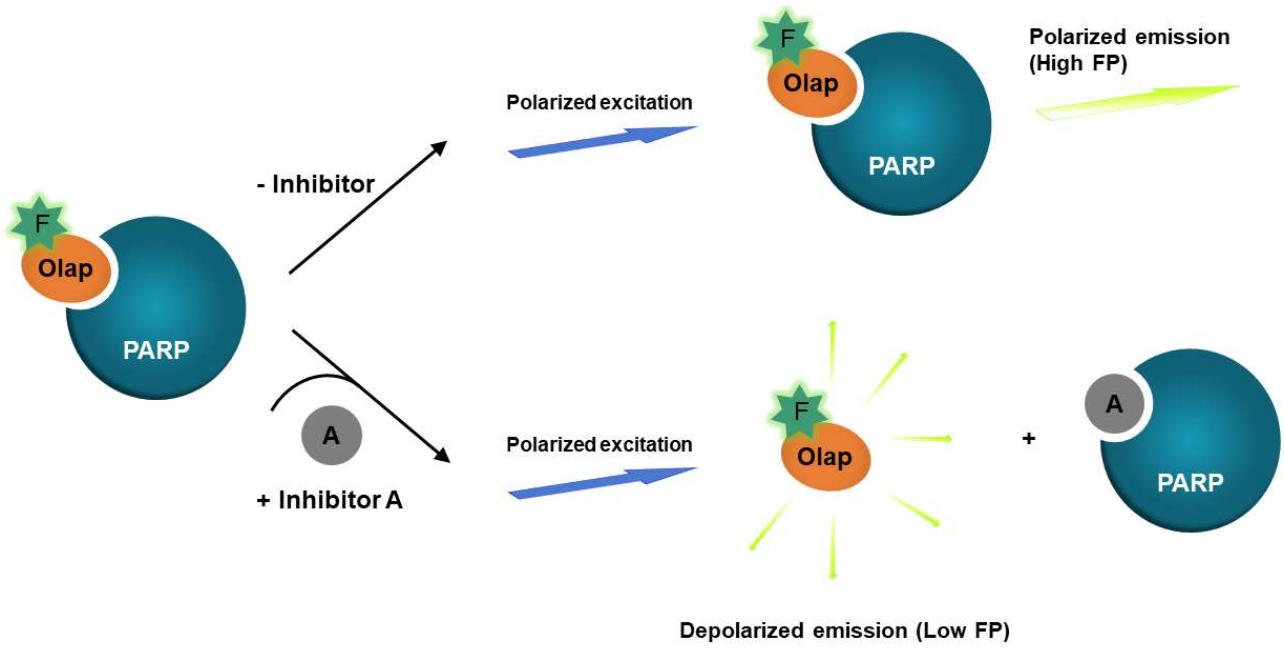

Figure 1: PARP1 Olaparib Competitive Inhibitor Assay Kit mechanism.

PARP1 binds to the Olaparib-containing fluorescent probe, forming a complex. This complex, when subjected to polarized excitation light, emits highly polarized light due to its restricted movement in solution. In the presence of a test compound (A), PARP1 may form a complex with either the test compound, if the compound has the same binding site of PARP1 as Olaparib, or with the Olaparib-containing fluorescent probe. If the test compound binds to PARP1 at the same site, the Olaparib-containing fluorescent probe remains in solution and rotates freely what is manifested by low FP. The decrease in FP value is proportional to the competitive binding of the test compound to PARP1.

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

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. PARP1 inhibitors have been used in cancer treatment with success. In addition to reducing MMEJ, the use of PARP1 inhibitors can lead to synthetic lethality when homologous recombination repair (HRR) mechanisms are already defective, as in the case of BRCA1 (breast cancer susceptibility protein type 1) and BRCA2 deficient cells. Further understanding of the molecular pathways involving PARP1, and this contribution to disease, will continue to pave the way for new therapies for PARP1-linked diseases.

Application(s)

- Screen molecules that bind to PARP1 in the same binding site as Olaparib for drug discovery high-throughput screening (HTS) applications.
- Determine IC_{50} values of PARP1 inhibitors with the same binding site as Olaparib.

Supplied Materials

Catalog #	Name	Amount	Storage
80501	PARP1, GST-Tag*	5 µg	-80°C
	10 µM PARPi-FL	5 µl	-80°C
	5x PARPtrap™ Assay Buffer 2	2 x 1 ml	-80°C
79685	96-well black microplate	1	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

- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- Fluorescent microplate reader capable of measuring fluorescence polarization (λex=485/20 nm and detection at λem=528/20 nm)

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

- This assay kit is compatible with up to 1% final DMSO concentration.
- Fluorescent compounds that have λex=485/20 nm and detection at λem=528/20 nm can interfere with the readouts.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include "Blank", "Reference Control" (minimum FP), "Positive Control" (maximum FP), and "Test Inhibitor" conditions.
- It is recommended all controls are run side by side as they may be necessary for result calculation.
- 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 Olaparib (#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 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).

1. Thaw **PARP1** and **PARPi-FL** on ice. Briefly spin the tubes to recover their full content.

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

3. Dilute PARP1 with 1x PARPtrap™ Assay Buffer 2 to 2.43 ng/µl (20 µl/well). The final concentration in the reaction is 7 nM.

4. 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.

   4.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x PARPtrap™ Assay Buffer 2, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x PARPtrap™ Assay Buffer 2 (Diluent Solution).

   OR

   4.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x PARPtrap™ Assay Buffer 2 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 1x PARPtrap™ Assay Buffer 2 to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in 1x PARPtrap™ Assay Buffer 2 (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. Add 20 µl of diluted PARP1 to "Positive Control" and "Test Inhibitor" wells.
6. Add 20 µl of 1x PARPtrap™ Assay Buffer 2 to "Reference Control" wells.
7. Add 45 µl of 1x PARPtrap™ Assay Buffer 2 to "Blank" wells.
8. Add 5 µl of Test Inhibitor solution to each well designated "Test Inhibitor".
9. Add 5 µl of Diluent Solution to the "Positive Control", "Reference Control", and "Blank" wells.
10. Dilute PARPi-FL in 1x PARPtrap™ Assay Buffer 2 to 6 nM (25 µl/well). The final concentration in the reaction is 3 nM.
11. Add 25 µl of diluted PARPi-FL to every well, except "Blank" wells.
12. Incubate at room temperature for 30-90 minutes with slow agitation.

Protect your samples from direct exposure to light. Photobleaching will occur!

Component	Test Inhibitor	Blank	Positive Control	Reference Control
Diluted PARP1 (2.43 ng/ µl)	20 µl	-	20 µl	-
1x PARPtrap™ Assay Buffer 2	-	45 µl	-	20 µl
Test Inhibitor	5 µl	–	–	-
Diluent Solution	–	5 µl	5 µl	5 µl
Diluted PARPi-FL (6 nM)	25 µl	-	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

13. Read FP in a fluorescence plate reader capable of measuring fluorescence polarization (λex = 485/20 nm; λem = 528/20 nm) **and set to FP**.
14. 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 ($I_{||}$) fluorescence intensity values emitted by the probe relative to the direction of the polarized excitation light (Figure 2).

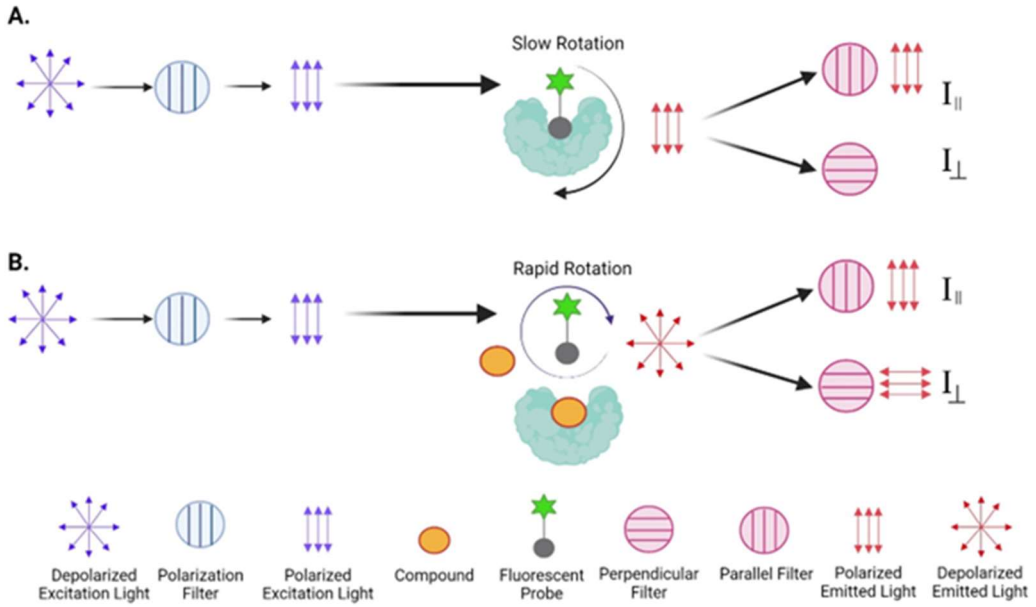

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Figure 2: Fluorescence polarization principle applied in FP assay kits.
A. When the fluorescently labeled probe binds to a larger protein it creates a complex of a big molecular weight that has a slow rotation ability. In this state the probe has a 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_{II}) 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).

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

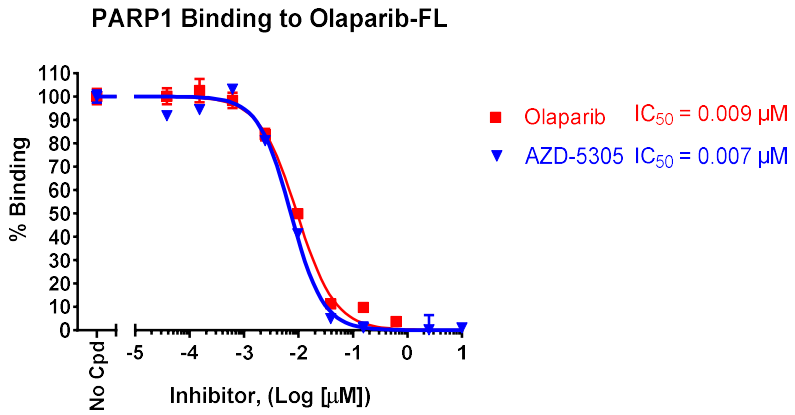

Figure 3: Competitive binding to PARP1 of Olaparib-containing fluorescent probe, Olaparib and AZD5305.
Binding of PARP1 to Olaparib-containing fluorescent probe was measured in the presence of increasing concentrations of Olaparib and AZD5305 (#78318). Results are expressed as percent of binding, in which binding in the absence of inhibitor is set to 100%.

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

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

References
Marques M., *et al.*, 2019 *Oncogene* 38 (12): 2177-2191.

Related Products

Products	*Catalog #*	*Size*
PARP1 Homogeneous Assay Kit	78438	384 reactions
PARP1 Colorimetric Assay Kit	80580	96 reactions
PARPtrap™ Assay Kit for PARP1	80560	96 reactions
PARP2 Chemiluminescent Assay Kit	80552	96 reactions
PARP3 Chemiluminescent Assay Kit	80553	96 reactions/384 reactions
PARP1 Chemiluminescent Assay Kit	80551	96 reactions

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