## Description

The PARPtrap™ Assay Kit for PARP2 is designed to measure PARP2/DNA complex formation in a high throughput screening assay using fluorescence polarization (FP). Similarly to PARP1, PARP2 recognizes and binds damaged DNA via its DNA-binding domain. Binding to DNA activates PARP2 and in the presence of NAD+ PARP2 ribosylates itself (auto-ribosylation), which 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 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 PARP2 enzyme comes in a convenient 384-well format, with purified PARP2 enzyme, fluorescent-labeled nicked oligonucleotide duplex, and PARPtrap™ assay buffer 2 for 400 enzyme reactions. The key to the PARPtrap™ Assay Kit is the fluorescent-labeled oligonucleotide duplex. In the absence of ribosylation, PARP2 binds to the fluorescent probe, forming a large complex and resulting in the emission of highly polarized light. However, after auto-ribosylation PARP2 dissociates from the oligonucleotide duplex, which then rotates freely, emitting less polarized light (Fig. 1). Addition of a PARP2 inhibitor results in trapping of PARP2 to the fluorescent oligonucleotide duplex, and increases the FP signal in a dose dependent manner.

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

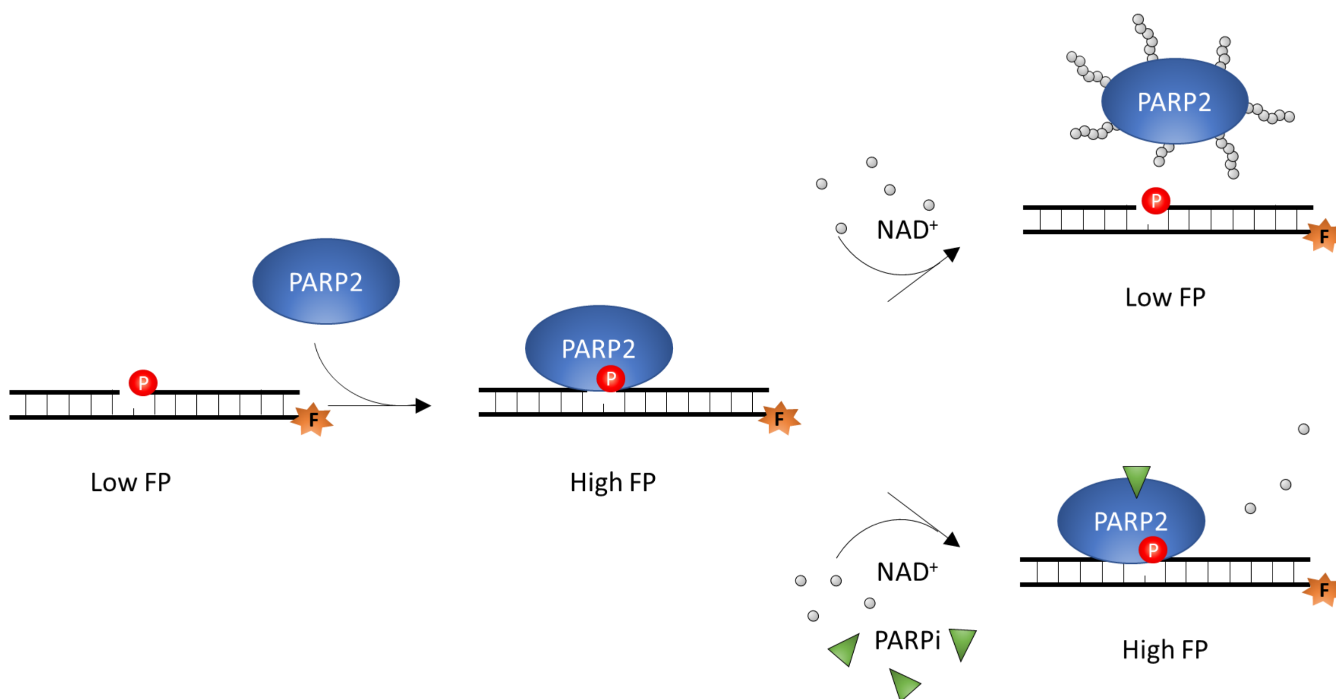


**Figure 1.** PARPtrap™ Assay Kit for PARP2 schematic

## Applications

Great for screening small molecules that enhance PARP2/DNA trapping in drug discovery and HTS applications, and for determination of compound $IC_{50}$.



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**Supplied Materials**

| Catalog # | Name | Amount | Storage | |
|---|---|---|---|---|
| 80502 | PARP2, GST-tag * | 2 x 8 µg | -80°C | ***Avoid multiple freeze/ thaw cycles*** |
| 78297 | Fluorescent labeled nicked oligonucleotide duplex (100 nM) | 2 x 12.5 µl | -80°C | |
| | 5x PARPtrap™ assay buffer 2 | 4 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: Kit updated on 08/02/2022. The revised kit now contains #78297 at concentration 100 nM and requires an added dilution step. Please verify the concentration of #78297 and make sure the corresponding datasheet is being used.*

**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™ Assay Kit for PARP2 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

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



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2) Dilute **100 nM Fluorescent labeled nicked DNA** 8-fold in distilled water to 12.5 nM**.** Dilute only enough required for the assay.

3) Prepare the Master Mix (10 µl per well): N wells x (3 µl of **5x PARPtrap™ assay buffer 2** (with DTT) + 0.5 µl of 1**2.5 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.

4) 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.

5) Prepare the test inhibitor solution.
If the 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 a diluent other than DMSO, use that diluent to prepare the control diluent solution.

6) Prepare the enzyme:
Thaw **PARP2** enzyme on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. 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.

7) 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 | 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 5 | 10 µl | 10 µl | 10 µl | – |
| Total | 22.5 µl | 22.5 µl | 22.5 µl | 22.5 µl |

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

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



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| | Blank |
|---|---|
| 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 PARP2, 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 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.
- Low FP control: a condition in which all 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 PARP2 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

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

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

11) Initiate PARP2 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.

12) 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.



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**Example Results**

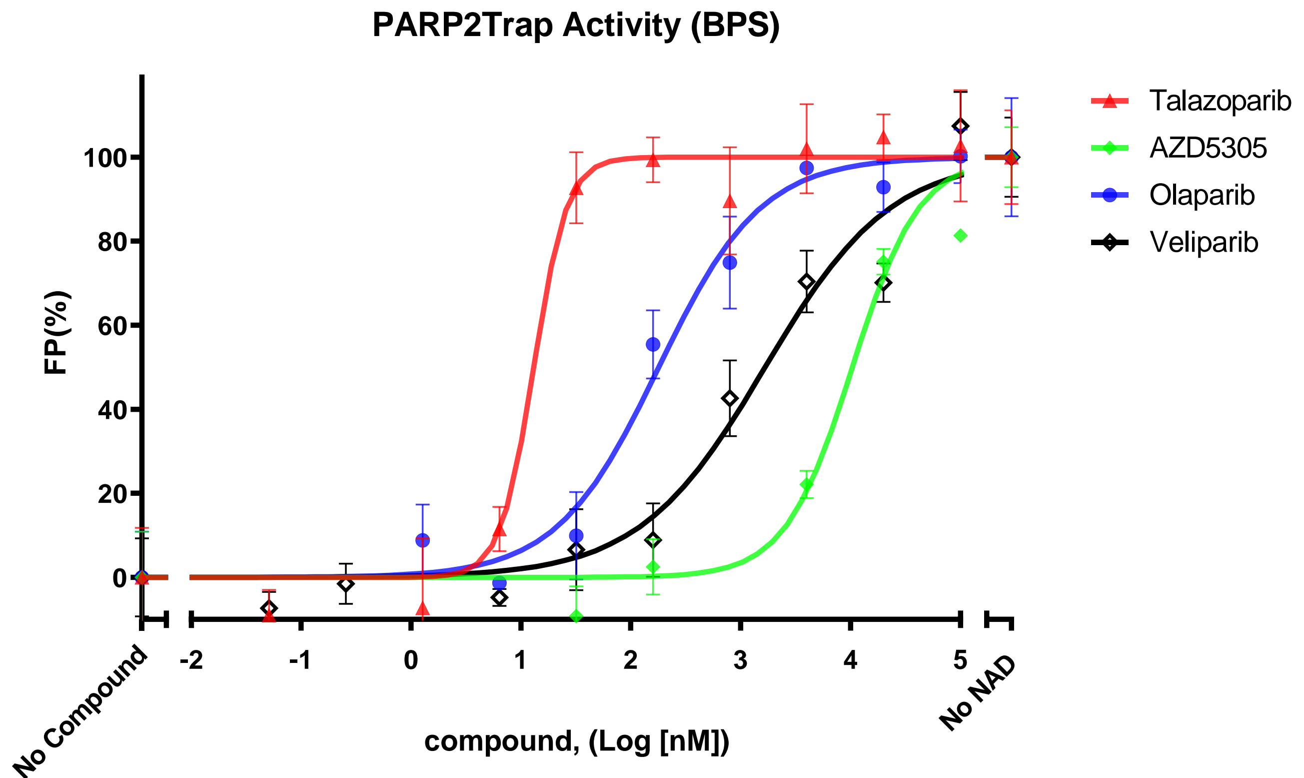


**Figure 1**: PARP2/DNA trapping measured in the presence of increasing concentrations of Talazoparib (Selleckchem), Olaparib (LC Laboratories), Veliparib (Selleckchem) and AZD5305 (MedChemExpress) using the PARPtrap™ Assay Kit for PARP2, BPS Bioscience, #78296. "No compound" corresponds to the "Low 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*

**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".

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

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




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**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| 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 |



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